

sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. See, e.g., Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (see, e.g., Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the NOVX expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerevisiae* include pYepSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, 1982. *Cell* 30: 933-943), pJRY88 (Schultz *et al.*, 1987. *Gene* 54: 113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (Invitrogen Corp, San Diego, Calif.).

Alternatively, NOVX can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., SF9 cells) include the pAc series (Smith, *et al.*, 1983. *Mol. Cell. Biol.* 3: 2156-2165) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, 1987. *Nature* 329: 840) and pMT2PC (Kaufman, *et al.*, 1987. *EMBO J.* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see, e.g., Chapters 16 and 17 of

Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert, *et al.*, 1987. *Genes Dev.* 1: 268-277), lymphoid-specific promoters (Calame and Eaton, 1988. *Adv. Immunol.* 43: 235-275), in particular promoters of T cell receptors (Winoto and Baltimore, 1989. *EMBO J.* 8: 729-733) and immunoglobulins (Banerji, *et al.*, 1983. *Cell* 33: 729-740; Queen and Baltimore, 1983. *Cell* 33: 741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle, 1989. *Proc. Natl. Acad. Sci. USA* 86: 5473-5477), pancreas-specific promoters (Edlund, *et al.*, 1985. *Science* 230: 912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss, 1990. *Science* 249: 374-379) and the  $\alpha$ -fetoprotein promoter (Campes and Tilghman, 1989. *Genes Dev.* 3: 537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively-linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to NOVX mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes *see, e.g.*, Weintraub, *et al.*, "Antisense RNA as a molecular tool for genetic analysis," *Reviews-Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer

not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

5           A host cell can be any prokaryotic or eukaryotic cell. For example, NOVX protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

10           Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in  
15   Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

20           For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding  
25   NOVX or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

30           A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) NOVX protein. Accordingly, the invention further provides methods for producing NOVX protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding NOVX protein has been introduced) in a suitable medium such that NOVX protein is produced. In another embodiment, the method further comprises isolating NOVX protein from the medium or the host cell.

### Transgenic NOVX Animals

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which NOVX protein-coding sequences have been introduced.

5 Such host cells can then be used to create non-human transgenic animals in which exogenous NOVX sequences have been introduced into their genome or homologous recombinant animals in which endogenous NOVX sequences have been altered. Such animals are useful for studying the function and/or activity of NOVX protein and for identifying and/or evaluating modulators of NOVX protein activity. As used herein, a "transgenic animal" is a  
10 non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and that remains in the genome of the mature  
15 animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous NOVX gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell  
20 of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing NOVX-encoding nucleic acid into the male pronuclei of a fertilized oocyte (*e.g.*, by microinjection, retroviral infection) and allowing the oocyte to develop in a pseudopregnant female foster animal. The human NOVX cDNA sequences SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27,  
25 29, and 31 can be introduced as a transgene into the genome of a non-human animal.

Alternatively, a non-human homologue of the human NOVX gene, such as a mouse NOVX gene, can be isolated based on hybridization to the human NOVX cDNA (described further *supra*) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A  
30 tissue-specific regulatory sequence(s) can be operably-linked to the NOVX transgene to direct expression of NOVX protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan, 1986. In: MANIPULATING THE MOUSE EMBRYO, Cold



Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the NOVX transgene in its genome and/or expression of NOVX mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed  
5 additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene-encoding NOVX protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of an NOVX gene into which a deletion, addition or substitution has been  
10 introduced to thereby alter, *e.g.*, functionally disrupt, the NOVX gene. The NOVX gene can be a human gene (*e.g.*, the cDNA of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, and 31), but more preferably, is a non-human homologue of a human NOVX gene. For example, a mouse homologue of human NOVX gene of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, and 31 can be used to construct a homologous recombination  
15 vector suitable for altering an endogenous NOVX gene in the mouse genome. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous NOVX gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the vector can be designed such that, upon homologous recombination,  
20 the endogenous NOVX gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous NOVX protein). In the homologous recombination vector, the altered portion of the NOVX gene is flanked at its 5'- and 3'-termini by additional nucleic acid of the NOVX gene to allow for homologous recombination to occur between the exogenous NOVX gene  
25 carried by the vector and an endogenous NOVX gene in an embryonic stem cell. The additional flanking NOVX nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5'- and 3'-termini) are included in the vector. *See, e.g.*, Thomas, *et al.*, 1987. *Cell* 51: 503 for a description of homologous recombination vectors. The vector is then introduced into  
30 an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced NOVX gene has homologously-recombined with the endogenous NOVX gene are selected. *See, e.g.*, Li, *et al.*, 1992. *Cell* 69: 915.

The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras. *See, e.g.*, Bradley, 1987. In: TERATOCARCINOMAS AND

EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152.

A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously-recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously-recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, 1991. *Curr. Opin. Biotechnol.* 2: 823-829; PCT International Publication Nos.: WO 90/11354; WO 91/01140; WO 92/0968; and WO 93/04169.

In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, *See, e.g.,* Lakso, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae*. *See, e.g.,* O'Gorman, *et al.*, 1991. *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.,* by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, *et al.*, 1997. *Nature* 385: 810-813. In brief, a cell (*e.g.,* a somatic cell) from the transgenic animal can be isolated and induced to exit the growth cycle and enter G<sub>0</sub> phase. The quiescent cell can then be fused, *e.g.,* through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell (*e.g.,* the somatic cell) is isolated.

### Pharmaceutical Compositions

The NOVX nucleic acid molecules, NOVX proteins, and anti-NOVX antibodies (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or

antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.*, topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol,

propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various  
5 antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum  
10 monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, an NOVX protein or anti-NOVX antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a  
15 sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

20 Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and  
25 swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a  
30 lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (*see, e.g.*, U.S. Patent No. 5,328,470) or by stereotactic injection (*see, e.g.*, Chen, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 3054-3057).

5 The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

10 The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

### Screening and Detection Methods

The isolated nucleic acid molecules of the invention can be used to express NOVX protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications),  
15 to detect NOVX mRNA (*e.g.*, in a biological sample) or a genetic lesion in an NOVX gene, and to modulate NOVX activity, as described further, below. In addition, the NOVX proteins can be used to screen drugs or compounds that modulate the NOVX protein activity or expression as well as to treat disorders characterized by insufficient or excessive production of NOVX protein or production of NOVX protein forms that have decreased or aberrant activity  
20 compared to NOVX wild-type protein (*e.g.*; diabetes (regulates insulin release); obesity (binds and transport lipids); metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers, and infectious disease (possesses anti-microbial activity) and the various dyslipidemias. In addition, the anti-NOVX antibodies of the invention can be used to detect  
25 and isolate NOVX proteins and modulate NOVX activity. In yet a further aspect, the invention can be used in methods to influence appetite, absorption of nutrients and the disposition of metabolic substrates in both a positive and negative fashion.

The invention further pertains to novel agents identified by the screening assays described herein and uses thereof for treatments as described, *supra*.

### Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides,

peptidomimetics, small molecules or other drugs) that bind to NOVX proteins or have a stimulatory or inhibitory effect on, *e.g.*, NOVX protein expression or NOVX protein activity. The invention also includes compounds identified in the screening assays described herein.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of the membrane-bound form of an NOVX protein or polypeptide or biologically-active portion thereof. The test compounds of the invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds. *See, e.g.*, Lam, 1997. *Anticancer Drug Design* 12: 145.

A "small molecule" as used herein, is meant to refer to a composition that has a molecular weight of less than about 5 kD and most preferably less than about 4 kD. Small molecules can be, *e.g.*, nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic or inorganic molecules. Libraries of chemical and/or biological mixtures, such as fungal, bacterial, or algal extracts, are known in the art and can be screened with any of the assays of the invention.

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt, *et al.*, 1993. *Proc. Natl. Acad. Sci. U.S.A.* 90: 6909; Erb, *et al.*, 1994. *Proc. Natl. Acad. Sci. U.S.A.* 91: 11422; Zuckermann, *et al.*, 1994. *J. Med. Chem.* 37: 2678; Cho, *et al.*, 1993. *Science* 261: 1303; Carrell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2059; Carell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2061; and Gallop, *et al.*, 1994. *J. Med. Chem.* 37: 1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten, 1992. *Biotechniques* 13: 412-421), or on beads (Lam, 1991. *Nature* 354: 82-84), on chips (Fodor, 1993. *Nature* 364: 555-556), bacteria (Ladner, U.S. Patent No. 5,223,409), spores (Ladner, U.S. Patent 5,233,409), plasmids (Cull, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 1865-1869) or on phage (Scott and Smith, 1990. *Science* 249: 386-390; Devlin, 1990. *Science* 249: 404-406; Cwirla, *et al.*, 1990. *Proc. Natl. Acad. Sci. U.S.A.* 87: 6378-6382; Felici, 1991. *J. Mol. Biol.* 222: 301-310; Ladner, U.S. Patent No. 5,233,409.).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of NOVX protein, or a biologically-active portion thereof, on the cell

surface is contacted with a test compound and the ability of the test compound to bind to an NOVX protein determined. The cell, for example, can be of mammalian origin or a yeast cell.

Determining the ability of the test compound to bind to the NOVX protein can be

accomplished, for example, by coupling the test compound with a radioisotope or enzymatic

5 label such that binding of the test compound to the NOVX protein or biologically-active

portion thereof can be determined by detecting the labeled compound in a complex. For

example, test compounds can be labeled with  $^{125}\text{I}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ , or  $^3\text{H}$ , either directly or indirectly,

and the radioisotope detected by direct counting of radioemission or by scintillation counting.

Alternatively, test compounds can be enzymatically-labeled with, for example, horseradish

10 peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by

determination of conversion of an appropriate substrate to product. In one embodiment, the

assay comprises contacting a cell which expresses a membrane-bound form of NOVX protein,

or a biologically-active portion thereof, on the cell surface with a known compound which

binds NOVX to form an assay mixture, contacting the assay mixture with a test compound,

15 and determining the ability of the test compound to interact with an NOVX protein, wherein

determining the ability of the test compound to interact with an NOVX protein comprises

determining the ability of the test compound to preferentially bind to NOVX protein or a

biologically-active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell

20 expressing a membrane-bound form of NOVX protein, or a biologically-active portion thereof,

on the cell surface with a test compound and determining the ability of the test compound to

modulate (*e.g.*, stimulate or inhibit) the activity of the NOVX protein or biologically-active

portion thereof. Determining the ability of the test compound to modulate the activity of

NOVX or a biologically-active portion thereof can be accomplished, for example, by

25 determining the ability of the NOVX protein to bind to or interact with an NOVX target

molecule. As used herein, a "target molecule" is a molecule with which an NOVX protein

binds or interacts in nature, for example, a molecule on the surface of a cell which expresses

an NOVX interacting protein, a molecule on the surface of a second cell, a molecule in the

extracellular milieu, a molecule associated with the internal surface of a cell membrane or a

30 cytoplasmic molecule. An NOVX target molecule can be a non-NOVX molecule or an

NOVX protein or polypeptide of the invention. In one embodiment, an NOVX target

molecule is a component of a signal transduction pathway that facilitates transduction of an

extracellular signal (*e.g.* a signal generated by binding of a compound to a membrane-bound

NOVX molecule) through the cell membrane and into the cell. The target, for example, can be



a second intercellular protein that has catalytic activity or a protein that facilitates the association of downstream signaling molecules with NOVX.

Determining the ability of the NOVX protein to bind to or interact with an NOVX target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the NOVX protein to bind to or interact with an NOVX target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular  $\text{Ca}^{2+}$ , diacylglycerol,  $\text{IP}_3$ , etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising an NOVX-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the invention is a cell-free assay comprising contacting an NOVX protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to bind to the NOVX protein or biologically-active portion thereof. Binding of the test compound to the NOVX protein can be determined either directly or indirectly as described above. In one such embodiment, the assay comprises contacting the NOVX protein or biologically-active portion thereof with a known compound which binds NOVX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an NOVX protein, wherein determining the ability of the test compound to interact with an NOVX protein comprises determining the ability of the test compound to preferentially bind to NOVX or biologically-active portion thereof as compared to the known compound.

In still another embodiment, an assay is a cell-free assay comprising contacting NOVX protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity of the NOVX protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of NOVX can be accomplished, for example, by determining the ability of the NOVX protein to bind to an NOVX target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of NOVX protein can be accomplished by determining the ability of the NOVX protein further modulate an NOVX target molecule. For

example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as described, *supra*.

In yet another embodiment, the cell-free assay comprises contacting the NOVX protein or biologically-active portion thereof with a known compound which binds NOVX protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an NOVX protein, wherein determining the ability of the test compound to interact with an NOVX protein comprises determining the ability of the NOVX protein to preferentially bind to or modulate the activity of an NOVX target molecule.

The cell-free assays of the invention are amenable to use of both the soluble form or the membrane-bound form of NOVX protein. In the case of cell-free assays comprising the membrane-bound form of NOVX protein, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of NOVX protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton<sup>®</sup> X-100, Triton<sup>®</sup> X-114, Thesit<sup>®</sup>, Isotridecypoly(ethylene glycol ether)<sub>n</sub>, N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-(3-cholamidopropyl) dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

In more than one embodiment of the above assay methods of the invention, it may be desirable to immobilize either NOVX protein or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to NOVX protein, or interaction of NOVX protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-NOVX fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or NOVX protein, and the mixture is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex

determined either directly or indirectly, for example, as described, *supra*. Alternatively, the complexes can be dissociated from the matrix, and the level of NOVX protein binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the NOVX protein or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated NOVX protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well-known within the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with NOVX protein or target molecules, but which do not interfere with binding of the NOVX protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or NOVX protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the NOVX protein or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the NOVX protein or target molecule.

In another embodiment, modulators of NOVX protein expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of NOVX mRNA or protein in the cell is determined. The level of expression of NOVX mRNA or protein in the presence of the candidate compound is compared to the level of expression of NOVX mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of NOVX mRNA or protein expression based upon this comparison. For example, when expression of NOVX mRNA or protein is greater (*i.e.*, statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of NOVX mRNA or protein expression. Alternatively, when expression of NOVX mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of NOVX mRNA or protein expression. The level of NOVX mRNA or protein expression in the cells can be determined by methods described herein for detecting NOVX mRNA or protein.

In yet another aspect of the invention, the NOVX proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos, *et al.*, 1993. *Cell* 72: 223-232; Madura, *et al.*, 1993. *J. Biol. Chem.* 268: 12046-12054;

Bartel, *et al.*, 1993. *Biotechniques* 14: 920-924; Iwabuchi, *et al.*, 1993. *Oncogene* 8: 1693-1696; and Brent WO 94/10300), to identify other proteins that bind to or interact with NOVX ("NOVX-binding proteins" or "NOVX-bp") and modulate NOVX activity. Such NOVX-binding proteins are also likely to be involved in the propagation of signals by the NOVX proteins as, for example, upstream or downstream elements of the NOVX pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for NOVX is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming an NOVX-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with NOVX.

The invention further pertains to novel agents identified by the aforementioned screening assays and uses thereof for treatments as described herein.

### Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. By way of example, and not of limitation, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. Some of these applications are described in the subsections, below.

### Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the NOVX sequences, SEQ ID

NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, and 31, or fragments or derivatives thereof, can be used to map the location of the NOVX genes, respectively, on a chromosome. The mapping of the NOVX sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

5 Briefly, NOVX genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the NOVX sequences. Computer analysis of the NOVX, sequences can be used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only  
10 those hybrids containing the human gene corresponding to the NOVX sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By  
15 using media in which mouse cells cannot grow, because they lack a particular enzyme, but in which human cells can, the one human chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy  
20 mapping of individual genes to specific human chromosomes. *See, e.g., D'Eustachio, et al., 1983. Science 220: 919-924.* Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular  
25 sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the NOVX sequences to design oligonucleotide primers, sub-localization can be achieved with panels of fragments from specific chromosomes.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase  
30 chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical like colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases.

However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases, will suffice to get good results at a reasonable amount of time. For a review of this technique, *see*, Verma, *et al.*, HUMAN CHROMOSOMES: A

5 MANUAL OF BASIC TECHNIQUES (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more  
10 likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, *e.g.*, in McKusick, MENDELIAN INHERITANCE IN MAN, available on-line  
15 through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, *e.g.*, Egeland, *et al.*, 1987. *Nature*, 325: 783-787.

Moreover, differences in the DNA sequences between individuals affected and  
20 unaffected with a disease associated with the NOVX gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome  
25 spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

### Tissue Typing

30 The NOVX sequences of the invention can also be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the invention are useful as additional DNA markers for RFLP ("restriction fragment length polymorphisms," described in U.S. Patent No. 5,272,057).

Furthermore, the sequences of the invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the NOVX sequences described herein can be used to prepare two PCR primers from the 5'- and 3'-termini of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the invention can be used to obtain such identification sequences from individuals and from tissue. The NOVX sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Much of the allelic variation is due to single nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, and 31 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

## **Predictive Medicine**

The invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the invention relates to diagnostic assays for determining NOVX protein and/or nucleic acid expression as well as NOVX activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant NOVX expression or activity. The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders,

Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with NOVX protein, nucleic acid expression or activity. For example, mutations in an NOVX gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with NOVX protein, nucleic acid expression, or biological activity.

Another aspect of the invention provides methods for determining NOVX protein, nucleic acid expression or activity in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (*e.g.*, drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (*e.g.*, the genotype of the individual examined to determine the ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of NOVX in clinical trials.

These and other agents are described in further detail in the following sections.

### **Diagnostic Assays**

An exemplary method for detecting the presence or absence of NOVX in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting NOVX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes NOVX protein such that the presence of NOVX is detected in the biological sample. An agent for detecting NOVX mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to NOVX mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length NOVX nucleic acid, such as the nucleic acid of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, and 31, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to NOVX mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.



An agent for detecting NOVX protein is an antibody capable of binding to NOVX protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')<sub>2</sub>) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass

5 direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary antibody and

10 end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect NOVX mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of NOVX mRNA include Northern hybridizations and *in situ*

15 hybridizations. *In vitro* techniques for detection of NOVX protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and immunofluorescence. *In vitro* techniques for detection of NOVX genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of NOVX protein include introducing into a subject a labeled anti-NOVX antibody. For example, the antibody

20 can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a

25 peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting NOVX protein, mRNA, or genomic DNA, such that the presence of NOVX protein, mRNA or genomic DNA is detected in the biological sample, and comparing

30 the presence of NOVX protein, mRNA or genomic DNA in the control sample with the presence of NOVX protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of NOVX in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting NOVX protein or mRNA in a biological sample; means for determining the amount

of NOVX in the sample; and means for comparing the amount of NOVX in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect NOVX protein or nucleic acid.

## 5           **Prognostic Assays**

          The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant NOVX expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk  
10 of developing a disorder associated with NOVX protein, nucleic acid expression or activity. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disease or disorder. Thus, the invention provides a method for identifying a disease or disorder associated with aberrant NOVX expression or activity in which a test sample is obtained from a subject and NOVX protein or nucleic acid (*e.g.*, mRNA, genomic  
15 DNA) is detected, wherein the presence of NOVX protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant NOVX expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

20           Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant NOVX expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder.  
25           Thus, the invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant NOVX expression or activity in which a test sample is obtained and NOVX protein or nucleic acid is detected (*e.g.*, wherein the presence of NOVX protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant NOVX expression or  
30 activity).

          The methods of the invention can also be used to detect genetic lesions in an NOVX gene, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized by aberrant cell proliferation and/or differentiation. In various embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a

genetic lesion characterized by at least one of an alteration affecting the integrity of a gene encoding an NOVX-protein, or the misexpression of the NOVX gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of: (i) a deletion of one or more nucleotides from an NOVX gene; (ii) an addition of one or more nucleotides to an NOVX gene; (iii) a substitution of one or more nucleotides of an NOVX gene, (iv) a chromosomal rearrangement of an NOVX gene; (v) an alteration in the level of a messenger RNA transcript of an NOVX gene, (vi) aberrant modification of an NOVX gene, such as of the methylation pattern of the genomic DNA, (vii) the presence of a non-wild-type splicing pattern of a messenger RNA transcript of an NOVX gene, (viii) a non-wild-type level of an NOVX protein, (ix) allelic loss of an NOVX gene, and (x) inappropriate post-translational modification of an NOVX protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in an NOVX gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (*see, e.g.,* U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (*see, e.g.,* Landegran, *et al.*, 1988. *Science* 241: 1077-1080; and Nakazawa, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 360-364), the latter of which can be particularly useful for detecting point mutations in the NOVX-gene (*see, Abravaya, et al.*, 1995. *Nucl. Acids Res.* 23: 675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g.,* genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to an NOVX gene under conditions such that hybridization and amplification of the NOVX gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (*see, Guatelli, et al.*, 1990. *Proc. Natl. Acad. Sci. USA* 87: 1874-1878), transcriptional amplification system (*see, Kwoh, et al.*, 1989. *Proc. Natl. Acad. Sci. USA* 86: 1173-1177); Q $\beta$  Replicase (*see, Lizardi, et al.*, 1988. *BioTechnology* 6: 1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to

those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in an NOVX gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (*see, e.g.*, U.S. Patent No. 5,493,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in NOVX can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high-density arrays containing hundreds or thousands of oligonucleotide probes. *See, e.g.*, Cronin, *et al.*, 1996. *Human Mutation* 7: 244-255; Kozal, *et al.*, 1996. *Nat. Med.* 2: 753-759. For example, genetic mutations in NOVX can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, *et al.*, *supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the NOVX gene and detect mutations by comparing the sequence of the sample NOVX with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert, 1977. *Proc. Natl. Acad. Sci. USA* 74: 560 or Sanger, 1977. *Proc. Natl. Acad. Sci. USA* 74: 5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (*see, e.g.*, Naeve, *et al.*, 1995. *Biotechniques* 19: 448), including sequencing by mass spectrometry (*see, e.g.*, PCT International Publication No. WO 94/16101; Cohen, *et al.*, 1996. *Adv. Chromatography* 36: 127-162; and Griffin, *et al.*, 1993. *Appl. Biochem. Biotechnol.* 38: 147-159).

Other methods for detecting mutations in the NOVX gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes. *See, e.g., Myers, et al., 1985. Science 230: 1242.* In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type NOVX sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S<sub>1</sub> nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. *See, e.g., Cotton, et al., 1988. Proc. Natl. Acad. Sci. USA 85: 4397; Saleeba, et al., 1992. Methods Enzymol. 217: 286-295.* In an embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in NOVX cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches. *See, e.g., Hsu, et al., 1994. Carcinogenesis 15: 1657-1662.* According to an exemplary embodiment, a probe based on an NOVX sequence, *e.g., a wild-type NOVX sequence*, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. *See, e.g., U.S. Patent No. 5,459,039.*

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in NOVX genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids. *See, e.g., Orita, et al., 1989. Proc. Natl. Acad. Sci. USA: 86: 2766; Cotton, 1993. Mutat. Res. 285: 125-144; Hayashi, 1992. Genet. Anal. Tech. Appl. 9: 73-79.* Single-stranded DNA fragments of sample and control NOVX nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection

of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility. *See, e.g., Keen, et al., 1991. Trends Genet. 7: 5.*

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE). *See, e.g., Myers, et al., 1985. Nature 313: 495.* When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA. *See, e.g., Rosenbaum and Reissner, 1987. Biophys. Chem. 265: 12753.*

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found. *See, e.g., Saiki, et al., 1986. Nature 324: 163; Saiki, et al., 1989. Proc. Natl. Acad. Sci. USA 86: 6230.* Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization; *see, e.g., Gibbs, et al., 1989. Nucl. Acids Res. 17: 2437-2448*) or at the extreme 3'-terminus of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (*see, e.g., Prossner, 1993. Tibtech. 11: 238*). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection. *See, e.g., Gasparini, et al., 1992. Mol. Cell Probes 6: 1.* It is anticipated that in certain embodiments amplification may also be performed using *Taq* ligase for amplification. *See, e.g., Barany, 1991. Proc. Natl. Acad. Sci. USA 88: 189.* In such cases, ligation will occur only if there is a

perfect match at the 3'-terminus of the 5' sequence, making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving an NOVX gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which NOVX is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

### Pharmacogenomics

Agents, or modulators that have a stimulatory or inhibitory effect on NOVX activity (*e.g.*, NOVX gene expression), as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.) In conjunction with such treatment, the pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (*e.g.*, drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of NOVX protein, expression of NOVX nucleic acid, or mutation content of NOVX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See

e.g., Eichelbaum, 1996. *Clin. Exp. Pharmacol. Physiol.*, 23: 983-985; Linder, 1997. *Clin. Chem.*, 43: 254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical complication is hemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. At the other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of NOVX protein, expression of NOVX nucleic acid, or mutation content of NOVX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an NOVX modulator, such as a modulator identified by one of the exemplary screening assays described herein.



### Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of NOVX (*e.g.*, the ability to modulate aberrant cell proliferation and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase NOVX gene expression, protein levels, or upregulate NOVX activity, can be monitored in clinical trials of subjects exhibiting decreased NOVX gene expression, protein levels, or downregulated NOVX activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease NOVX gene expression, protein levels, or downregulate NOVX activity, can be monitored in clinical trials of subjects exhibiting increased NOVX gene expression, protein levels, or upregulated NOVX activity. In such clinical trials, the expression or activity of NOVX and, preferably, other genes that have been implicated in, for example, a cellular proliferation or immune disorder can be used as a "read out" or markers of the immune responsiveness of a particular cell.

By way of example, and not of limitation, genes, including NOVX, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates NOVX activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of NOVX and other genes implicated in the disorder. The levels of gene expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of NOVX or other genes. In this manner, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In one embodiment, the invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, protein, peptide, peptidomimetic, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of an NOVX protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of

expression or activity of the NOVX protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the NOVX protein, mRNA, or genomic DNA in the pre-administration sample with the NOVX protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of NOVX to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of NOVX to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

## Methods of Treatment

The invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant NOVX expression or activity. The disorders include cardiomyopathy, atherosclerosis, hypertension, congenital heart defects, aortic stenosis, atrial septal defect (ASD), atrioventricular (A-V) canal defect, ductus arteriosus, pulmonary stenosis, subaortic stenosis, ventricular septal defect (VSD), valve diseases, tuberous sclerosis, scleroderma, obesity, transplantation, adrenoleukodystrophy, congenital adrenal hyperplasia, prostate cancer, neoplasm; adenocarcinoma, lymphoma, uterus cancer, fertility, hemophilia, hypercoagulation, idiopathic thrombocytopenic purpura, immunodeficiencies, graft versus host disease, AIDS, bronchial asthma, Crohn's disease; multiple sclerosis, treatment of Albright Hereditary Osteodystrophy, and other diseases, disorders and conditions of the like.

These methods of treatment will be discussed more fully, below.

## Disease and Disorders

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Therapeutics that antagonize activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to: (i) an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; (ii) antibodies to an aforementioned peptide; (iii) nucleic acids encoding an aforementioned peptide; (iv) administration of antisense nucleic acid and nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of coding sequences to an aforementioned peptide) that are utilized to

"knockout" endogenous function of an aforementioned peptide by homologous recombination (see, e.g., Capecchi, 1989. *Science* 244: 1288-1292); or (v) modulators (i.e., inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between an aforementioned peptide and its binding partner.

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that increase (i.e., are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (e.g., from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of an aforementioned peptide). Methods that are well-known within the art include, but are not limited to, immunoassays (e.g., by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (e.g., Northern assays, dot blots, *in situ* hybridization, and the like).

### **Prophylactic Methods**

In one aspect, the invention provides a method for preventing, in a subject, a disease or condition associated with an aberrant NOVX expression or activity, by administering to the subject an agent that modulates NOVX expression or at least one NOVX activity. Subjects at risk for a disease that is caused or contributed to by aberrant NOVX expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the NOVX aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending upon the type of NOVX aberrancy, for example, an NOVX agonist or NOVX antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein. The prophylactic methods of the invention are further discussed in the following subsections.

### Therapeutic Methods

Another aspect of the invention pertains to methods of modulating NOVX expression or activity for therapeutic purposes. The modulatory method of the invention involves

5 contacting a cell with an agent that modulates one or more of the activities of NOVX protein activity associated with the cell. An agent that modulates NOVX protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of an NOVX protein, a peptide, an NOVX peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more NOVX protein activity. Examples of such

10 stimulatory agents include active NOVX protein and a nucleic acid molecule encoding NOVX that has been introduced into the cell. In another embodiment, the agent inhibits one or more NOVX protein activity. Examples of such inhibitory agents include antisense NOVX nucleic acid molecules and anti-NOVX antibodies. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering

15 the agent to a subject). As such, the invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of an NOVX protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., up-regulates or down-regulates) NOVX expression

20 or activity. In another embodiment, the method involves administering an NOVX protein or nucleic acid molecule as therapy to compensate for reduced or aberrant NOVX expression or activity.

Stimulation of NOVX activity is desirable *in situations* in which NOVX is abnormally downregulated and/or in which increased NOVX activity is likely to have a beneficial effect.

25 One example of such a situation is where a subject has a disorder characterized by aberrant cell proliferation and/or differentiation (e.g., cancer or immune associated disorders). Another example of such a situation is where the subject has a gestational disease (e.g., preclampsia).

### Determination of the Biological Effect of the Therapeutic

In various embodiments of the invention, suitable *in vitro* or *in vivo* assays are

30 performed to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue.

In various specific embodiments, *in vitro* assays may be performed with representative cells of the type(s) involved in the patient's disorder, to determine if a given Therapeutic exerts

the desired effect upon the cell type(s). Compounds for use in therapy may be tested in suitable animal model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in human subjects. Similarly, for *in vivo* testing, any of the animal model system known in the art may be used prior to administration to human subjects.

### **Prophylactic and Therapeutic Uses of the Compositions of the Invention**

The NOVX nucleic acids and proteins of the invention are useful in potential prophylactic and therapeutic applications implicated in a variety of disorders including, but not limited to: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.

As an example, a cDNA encoding the NOVX protein of the invention may be useful in gene therapy, and the protein may be useful when administered to a subject in need thereof. By way of non-limiting example, the compositions of the invention will have efficacy for treatment of patients suffering from: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias.

Both the novel nucleic acid encoding the NOVX protein, and the NOVX protein of the invention, or fragments thereof, may also be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. A further use could be as an anti-bacterial molecule (*i.e.*, some peptides have been found to possess anti-bacterial properties). These materials are further useful in the generation of antibodies, which immunospecifically-bind to the novel substances of the invention for use in therapeutic or diagnostic methods.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

## Examples

### Example 1. Identification of NOVX clones

The novel NOVX target sequences identified in the present invention were subjected to the exon linking process to confirm the sequence. PCR primers were designed by starting at the most upstream sequence available, for the forward primer, and at the most downstream sequence available for the reverse primer. Table 13A shows the sequences of the PCR primers used for obtaining different clones. In each case, the sequence was examined, walking inward from the respective termini toward the coding sequence, until a suitable sequence that is either unique or highly selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such primers were designed based on in silico predictions for the full length cDNA, part (one or more exons) of the DNA or protein sequence of the target sequence, or by translated homology of the predicted exons to closely related human sequences from other species. These primers were then employed in PCR amplification based on the following pool of human cDNAs: adrenal gland, bone marrow, brain - amygdala, brain - cerebellum, brain - hippocampus, brain - substantia nigra, brain - thalamus, brain - whole, fetal brain, fetal kidney, fetal liver, fetal lung, heart, kidney, lymphoma - Raji, mammary gland, pancreas, pituitary gland, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thyroid, trachea, uterus. Usually the resulting amplicons were gel purified, cloned and sequenced to high redundancy. The PCR product derived from exon linking was cloned into the pCR2.1 vector from Invitrogen. The resulting bacterial clone has an insert covering the entire open reading frame cloned into the pCR2.1 vector. Table 13B shows a list of these bacterial clones. The resulting sequences from all clones were assembled with themselves, with other fragments in CuraGen Corporation's database and with public ESTs. Fragments and ESTs were included as components for an assembly when the extent of their identity with another component of the assembly was at least 95% over 50 bp. In addition, sequence traces were evaluated manually and edited for corrections if appropriate. These procedures provide the sequence reported herein.

**Table 13A. PCR Primers for Exon Linking**

| NOVX Clone | Primer 1 (5' - 3')    | SEQ ID NO | Primer 2 (5' - 3')           | SEQ ID NO |
|------------|-----------------------|-----------|------------------------------|-----------|
| NOV1a      | AGACTGGGGCCAGGGAGACAG | 119       | CAGAGGCCAAACATCCCCATCAG      | 120       |
| NOV1c      | GAGACAGCCCTGGGGGAGA   | 121       | ACCTGCCTCCTGCCAGTCC          | 122       |
| NOV6       | CATGTCCTCGACCGAGAGCGC | 123       | AGGTGGGGGGCTGCTTACTGCTT      | 124       |
| NOV9a      | GTCATGAAGGGGTTGCTG    | 125       | GGTCAGCCCAGCCCCTCTG          | 126       |
| NOV10      | CGGCTGCTGGCATGGGTG    | 127       | CTCCTGCTCTGTTTCCCCCTTCAT     | 128       |
| NOV11a     | GCCATGGTGCTGCTGCTGCT  | 129       | GGCTCAGTCGGGGTAGATGATAAAGC   | 130       |
| NOV11b     | CGGGCGCGGCCGTCGGAGT   | 131       | CGGGCGCGGCTCAGTCGGGGTAGATGAT | 132       |

Physical clone: Exons were predicted by homology and the intron/exon boundaries were determined using standard genetic rules. Exons were further selected and refined by means of similarity determination using multiple BLAST (for example, tBlastN, BlastX, and BlastN) searches, and, in some instances, GeneScan and Grail. Expressed sequences from both public and proprietary databases were also added when available to further define and complete the gene sequence. The DNA sequence was then manually corrected for apparent inconsistencies thereby obtaining the sequences encoding the full-length protein.

**Table 13B. Physical Clones for PCR products**

| NOVX Clone | Clone                                                                                           |
|------------|-------------------------------------------------------------------------------------------------|
| NOV1a      | Proprietary clones: 145150175, 145150395, 145150392, 145145203, 145150171, 145150168, 137114011 |
| NOV2a      | Physical clones: 107029754, AC078825, AC083812                                                  |
| NOV3       | Physical clones: 134899552, AC005230                                                            |
| NOV4       | Genomic clone: ba568g11                                                                         |
| NOV5       | Genomic clone: AC008774                                                                         |
| NOV6       | Bacterial clone: 111865::GMAC073364 A.698299.A2                                                 |
| NOV7       | Physical clone: 106973211, AC015855.4                                                           |
| NOV8       | Physical clone: 88091010, AL109932.3, AL360269.3, AL356323.6                                    |
| NOV10      | Proprietary clones: 140488852, 133419352, 141920635                                             |
| NOV11a     | Genomic clone: AC026125                                                                         |
| NOV12      | Genomic clone: AC011199                                                                         |

**Example 2. Quantitative expression analysis of clones in various tissues and cells**

The quantitative expression of various clones was assessed using microtiter plates containing RNA samples from a variety of normal and pathology-derived cells, cell lines and tissues using real time quantitative PCR (RTQ PCR). RTQ PCR was performed on an Applied Biosystems ABI PRISM® 7700 or an ABI PRISM® 7900 HT Sequence Detection System. Various collections of samples are assembled on the plates, and referred to as Panel 1 (containing normal tissues and cancer cell lines), Panel 2 (containing samples derived from tissues from normal and cancer sources), Panel 3 (containing cancer cell lines), Panel 4 (containing cells and cell lines from normal tissues and cells related to inflammatory conditions), Panel 5D/5I (containing human tissues and cell lines with an emphasis on metabolic diseases), AI\_comprehensive\_panel (containing normal tissue and samples from autoinflammatory diseases), Panel CNSD.01 (containing samples from normal and diseased brains) and CNS\_neurodegeneration\_panel (containing samples from normal and Alzheimer's diseased brains).

RNA integrity from all samples is controlled for quality by visual assessment of agarose gel electropherograms using 28S and 18S ribosomal RNA staining intensity ratio as a guide (2:1 to 2.5:1 28s:18s) and the absence of low molecular weight RNAs that would be

indicative of degradation products. Samples are controlled against genomic DNA contamination by RTQ PCR reactions run in the absence of reverse transcriptase using probe and primer sets designed to amplify across the span of a single exon.

First, the RNA samples were normalized to reference nucleic acids such as constitutively expressed genes (for example,  $\beta$ -actin and GAPDH). Normalized RNA (5  $\mu$ l) was converted to cDNA and analyzed by RTQ-PCR using One Step RT-PCR Master Mix Reagents (Applied Biosystems; Catalog No. 4309169) and gene-specific primers according to the manufacturer's instructions.

In other cases, non-normalized RNA samples were converted to single strand cDNA (sscDNA) using Superscript II (Invitrogen Corporation; Catalog No. 18064-147) and random hexamers according to the manufacturer's instructions. Reactions containing up to 10  $\mu$ g of total RNA were performed in a volume of 20  $\mu$ l and incubated for 60 minutes at 42°C. This reaction can be scaled up to 50  $\mu$ g of total RNA in a final volume of 100  $\mu$ l. sscDNA samples are then normalized to reference nucleic acids as described previously, using 1X TaqMan® Universal Master mix (Applied Biosystems; catalog No. 4324020), following the manufacturer's instructions.

Probes and primers were designed for each assay according to Applied Biosystems Primer Express Software package (version I for Apple Computer's Macintosh Power PC) or a similar algorithm using the target sequence as input. Default settings were used for reaction conditions and the following parameters were set before selecting primers: primer concentration = 250 nM, primer melting temperature ( $T_m$ ) range = 58°-60°C, primer optimal  $T_m$  = 59°C, maximum primer difference = 2°C, probe does not have 5'G, probe  $T_m$  must be 10°C greater than primer  $T_m$ , amplicon size 75bp to 100bp. The probes and primers selected (see below) were synthesized by SyntheGen (Houston, TX, USA). Probes were double purified by HPLC to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: forward and reverse primers, 900nM each, and probe, 200nM.

PCR conditions: When working with RNA samples, normalized RNA from each tissue and each cell line was spotted in each well of either a 96 well or a 384-well PCR plate (Applied Biosystems). PCR cocktails included either a single gene specific probe and primers set, or two multiplexed probe and primers sets (a set specific for the target clone and another gene-specific set multiplexed with the target probe). PCR reactions were set up using TaqMan® One-Step RT-PCR Master Mix (Applied Biosystems, Catalog No. 4313803)



following manufacturer's instructions. Reverse transcription was performed at 48°C for 30 minutes followed by amplification/PCR cycles as follows: 95°C 10 min, then 40 cycles of 95°C for 15 seconds, 60°C for 1 minute. Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) using a log scale, with the difference in RNA concentration between a given sample and the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression is then obtained by taking the reciprocal of this RNA difference and multiplying by 100.

When working with sscDNA samples, normalized sscDNA was used as described previously for RNA samples. PCR reactions containing one or two sets of probe and primers were set up as described previously, using 1X TaqMan® Universal Master mix (Applied Biosystems; catalog No. 4324020), following the manufacturer's instructions. PCR amplification was performed as follows: 95°C 10 min, then 40 cycles of 95°C for 15 seconds, 60°C for 1 minute. Results were analyzed and processed as described previously.

#### **Panels 1, 1.1, 1.2, and 1.3D**

The plates for Panels 1, 1.1, 1.2 and 1.3D include 2 control wells (genomic DNA control and chemistry control) and 94 wells containing cDNA from various samples. The samples in these panels are broken into 2 classes: samples derived from cultured cell lines and samples derived from primary normal tissues. The cell lines are derived from cancers of the following types: lung cancer, breast cancer, melanoma, colon cancer, prostate cancer, CNS cancer, squamous cell carcinoma, ovarian cancer, liver cancer, renal cancer, gastric cancer and pancreatic cancer. Cell lines used in these panels are widely available through the American Type Culture Collection (ATCC), a repository for cultured cell lines, and were cultured using the conditions recommended by the ATCC. The normal tissues found on these panels are comprised of samples derived from all major organ systems from single adult individuals or fetuses. These samples are derived from the following organs: adult skeletal muscle, fetal skeletal muscle, adult heart, fetal heart, adult kidney, fetal kidney, adult liver, fetal liver, adult lung, fetal lung, various regions of the brain, the spleen, bone marrow, lymph node, pancreas, salivary gland, pituitary gland, adrenal gland, spinal cord, thymus, stomach, small intestine, colon, bladder, trachea, breast, ovary, uterus, placenta, prostate, testis and adipose.

In the results for Panels 1, 1.1, 1.2 and 1.3D, the following abbreviations are used:

ca. = carcinoma,  
\* = established from metastasis,  
met = metastasis,  
s cell var = small cell variant,

non-s = non-sm = non-small,  
squamous = squamous,  
pl. eff = pl effusion = pleural effusion,  
glio = glioma,  
astro = astrocytoma, and  
neuro = neuroblastoma.

#### **General\_screening\_panel\_v1.4**

The plates for Panel 1.4 include 2 control wells (genomic DNA control and chemistry control) and 94 wells containing cDNA from various samples. The samples in Panel 1.4 are broken into 2 classes: samples derived from cultured cell lines and samples derived from primary normal tissues. The cell lines are derived from cancers of the following types: lung cancer, breast cancer, melanoma, colon cancer, prostate cancer, CNS cancer, squamous cell carcinoma, ovarian cancer, liver cancer, renal cancer, gastric cancer and pancreatic cancer.

Cell lines used in Panel 1.4 are widely available through the American Type Culture Collection (ATCC), a repository for cultured cell lines, and were cultured using the conditions recommended by the ATCC. The normal tissues found on Panel 1.4 are comprised of pools of samples derived from all major organ systems from 2 to 5 different adult individuals or fetuses. These samples are derived from the following organs: adult skeletal muscle, fetal skeletal muscle, adult heart, fetal heart, adult kidney, fetal kidney, adult liver, fetal liver, adult lung, fetal lung, various regions of the brain, the spleen, bone marrow, lymph node, pancreas, salivary gland, pituitary gland, adrenal gland, spinal cord, thymus, stomach, small intestine, colon, bladder, trachea, breast, ovary, uterus, placenta, prostate, testis and adipose. Abbreviations are as described for Panels 1, 1.1, 1.2, and 1.3D.

#### **Panels 2D and 2.2**

The plates for Panels 2D and 2.2 generally include 2 control wells and 94 test samples composed of RNA or cDNA isolated from human tissue procured by surgeons working in close cooperation with the National Cancer Institute's Cooperative Human Tissue Network (CHTN) or the National Disease Research Initiative (NDRI). The tissues are derived from human malignancies and in cases where indicated many malignant tissues have "matched margins" obtained from noncancerous tissue just adjacent to the tumor. These are termed normal adjacent tissues and are denoted "NAT" in the results below. The tumor tissue and the "matched margins" are evaluated by two independent pathologists (the surgical pathologists and again by a pathologist at NDRI or CHTN). This analysis provides a gross histopathological assessment of tumor differentiation grade. Moreover, most samples include

the original surgical pathology report that provides information regarding the clinical stage of the patient. These matched margins are taken from the tissue surrounding (i.e. immediately proximal) to the zone of surgery (designated "NAT", for normal adjacent tissue, in Table RR). In addition, RNA and cDNA samples were obtained from various human tissues derived from autopsies performed on elderly people or sudden death victims (accidents, etc.). These tissues were ascertained to be free of disease and were purchased from various commercial sources such as Clontech (Palo Alto, CA), Research Genetics, and Invitrogen.

### Panel 3D

The plates of Panel 3D are comprised of 94 cDNA samples and two control samples. Specifically, 92 of these samples are derived from cultured human cancer cell lines, 2 samples of human primary cerebellar tissue and 2 controls. The human cell lines are generally obtained from ATCC (American Type Culture Collection), NCI or the German tumor cell bank and fall into the following tissue groups: Squamous cell carcinoma of the tongue, breast cancer, prostate cancer, melanoma, epidermoid carcinoma, sarcomas, bladder carcinomas, pancreatic cancers, kidney cancers, leukemias/lymphomas, ovarian/uterine/cervical, gastric, colon, lung and CNS cancer cell lines. In addition, there are two independent samples of cerebellum. These cells are all cultured under standard recommended conditions and RNA extracted using the standard procedures. The cell lines in panel 3D and 1.3D are of the most common cell lines used in the scientific literature.

### Panels 4D, 4R, and 4.1D

Panel 4 includes samples on a 96 well plate (2 control wells, 94 test samples) composed of RNA (Panel 4R) or cDNA (Panels 4D/4.1D) isolated from various human cell lines or tissues related to inflammatory conditions. Total RNA from control normal tissues such as colon and lung (Stratagene, La Jolla, CA) and thymus and kidney (Clontech) was employed. Total RNA from liver tissue from cirrhosis patients and kidney from lupus patients was obtained from BioChain (Biochain Institute, Inc., Hayward, CA). Intestinal tissue for RNA preparation from patients diagnosed as having Crohn's disease and ulcerative colitis was obtained from the National Disease Research Interchange (NDRI) (Philadelphia, PA).

Astrocytes, lung fibroblasts, dermal fibroblasts, coronary artery smooth muscle cells, small airway epithelium, bronchial epithelium, microvascular dermal endothelial cells, microvascular lung endothelial cells, human pulmonary aortic endothelial cells, human umbilical vein endothelial cells were all purchased from Clonetics (Walkersville, MD) and grown in the media supplied for these cell types by Clonetics. These primary cell types were

activated with various cytokines or combinations of cytokines for 6 and/or 12-14 hours, as indicated. The following cytokines were used; IL-1 beta at approximately 1-5ng/ml, TNF alpha at approximately 5-10ng/ml, IFN gamma at approximately 20-50ng/ml, IL-4 at approximately 5-10ng/ml, IL-9 at approximately 5-10ng/ml, IL-13 at approximately 5-10ng/ml. Endothelial cells were sometimes starved for various times by culture in the basal media from Clonetics with 0.1% serum.

Mononuclear cells were prepared from blood of employees at CuraGen Corporation, using Ficoll. LAK cells were prepared from these cells by culture in DMEM 5% FCS (Hyclone), 100μM non essential amino acids (Gibco/Life Technologies, Rockville, MD), 1mM sodium pyruvate (Gibco), mercaptoethanol  $5.5 \times 10^{-5}$ M (Gibco), and 10mM Hepes (Gibco) and Interleukin 2 for 4-6 days. Cells were then either activated with 10-20ng/ml PMA and 1-2μg/ml ionomycin, IL-12 at 5-10ng/ml, IFN gamma at 20-50ng/ml and IL-18 at 5-10ng/ml for 6 hours. In some cases, mononuclear cells were cultured for 4-5 days in DMEM 5% FCS (Hyclone), 100μM non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol  $5.5 \times 10^{-5}$ M (Gibco), and 10mM Hepes (Gibco) with PHA (phytohemagglutinin) or PWM (pokeweed mitogen) at approximately 5μg/ml. Samples were taken at 24, 48 and 72 hours for RNA preparation. MLR (mixed lymphocyte reaction) samples were obtained by taking blood from two donors, isolating the mononuclear cells using Ficoll and mixing the isolated mononuclear cells 1:1 at a final concentration of approximately  $2 \times 10^6$  cells/ml in DMEM 5% FCS (Hyclone), 100μM non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol ( $5.5 \times 10^{-5}$ M) (Gibco), and 10mM Hepes (Gibco). The MLR was cultured and samples taken at various time points ranging from 1- 7 days for RNA preparation.

Monocytes were isolated from mononuclear cells using CD14 Miltenyi Beads, +ve VS selection columns and a Vario Magnet according to the manufacturer's instructions. Monocytes were differentiated into dendritic cells by culture in DMEM 5% fetal calf serum (FCS) (Hyclone, Logan, UT), 100μM non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol  $5.5 \times 10^{-5}$ M (Gibco), and 10mM Hepes (Gibco), 50ng/ml GM-CSF and 5ng/ml IL-4 for 5-7 days. Macrophages were prepared by culture of monocytes for 5-7 days in DMEM 5% FCS (Hyclone), 100μM non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol  $5.5 \times 10^{-5}$ M (Gibco), 10mM Hepes (Gibco) and 10% AB Human Serum or MCSF at approximately 50ng/ml. Monocytes, macrophages and dendritic cells were stimulated for 6 and 12-14 hours with lipopolysaccharide (LPS) at

100ng/ml. Dendritic cells were also stimulated with anti-CD40 monoclonal antibody (Pharmingen) at 10µg/ml for 6 and 12-14 hours.

CD4 lymphocytes, CD8 lymphocytes and NK cells were also isolated from mononuclear cells using CD4, CD8 and CD56 Miltenyi beads, positive VS selection columns and a Vario Magnet according to the manufacturer's instructions. CD45RA and CD45RO CD4 lymphocytes were isolated by depleting mononuclear cells of CD8, CD56, CD14 and CD19 cells using CD8, CD56, CD14 and CD19 Miltenyi beads and positive selection. CD45RO beads were then used to isolate the CD45RO CD4 lymphocytes with the remaining cells being CD45RA CD4 lymphocytes. CD45RA CD4, CD45RO CD4 and CD8 lymphocytes were placed in DMEM 5% FCS (Hyclone), 100µM non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol  $5.5 \times 10^{-5}$ M (Gibco), and 10mM Hepes (Gibco) and plated at  $10^6$  cells/ml onto Falcon 6 well tissue culture plates that had been coated overnight with 0.5µg/ml anti-CD28 (Pharmingen) and 3µg/ml anti-CD3 (OKT3, ATCC) in PBS. After 6 and 24 hours, the cells were harvested for RNA preparation. To prepare chronically activated CD8 lymphocytes, we activated the isolated CD8 lymphocytes for 4 days on anti-CD28 and anti-CD3 coated plates and then harvested the cells and expanded them in DMEM 5% FCS (Hyclone), 100µM non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol  $5.5 \times 10^{-5}$ M (Gibco), and 10mM Hepes (Gibco) and IL-2. The expanded CD8 cells were then activated again with plate bound anti-CD3 and anti-CD28 for 4 days and expanded as before. RNA was isolated 6 and 24 hours after the second activation and after 4 days of the second expansion culture. The isolated NK cells were cultured in DMEM 5% FCS (Hyclone), 100µM non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol  $5.5 \times 10^{-5}$ M (Gibco), and 10mM Hepes (Gibco) and IL-2 for 4-6 days before RNA was prepared.

To obtain B cells, tonsils were procured from NDRI. The tonsil was cut up with sterile dissecting scissors and then passed through a sieve. Tonsil cells were then spun down and resuspended at  $10^6$  cells/ml in DMEM 5% FCS (Hyclone), 100µM non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol  $5.5 \times 10^{-5}$ M (Gibco), and 10mM Hepes (Gibco). To activate the cells, we used PWM at 5µg/ml or anti-CD40 (Pharmingen) at approximately 10µg/ml and IL-4 at 5-10ng/ml. Cells were harvested for RNA preparation at 24, 48 and 72 hours.

To prepare the primary and secondary Th1/Th2 and Tr1 cells, six-well Falcon plates were coated overnight with 10µg/ml anti-CD28 (Pharmingen) and 2µg/ml OKT3 (ATCC), and then washed twice with PBS. Umbilical cord blood CD4 lymphocytes (Poietic Systems,

German Town, MD) were cultured at  $10^5$ - $10^6$  cells/ml in DMEM 5% FCS (Hyclone), 100 $\mu$ M non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol  $5.5 \times 10^{-5}$  M (Gibco), 10mM Hepes (Gibco) and IL-2 (4ng/ml). IL-12 (5ng/ml) and anti-IL4 (1 $\mu$ g/ml) were used to direct to Th1, while IL-4 (5ng/ml) and anti-IFN gamma (1 $\mu$ g/ml) were used to direct to Th2 and IL-10 at 5ng/ml was used to direct to Tr1. After 4-5 days, the activated Th1, Th2 and Tr1 lymphocytes were washed once in DMEM and expanded for 4-7 days in DMEM 5% FCS (Hyclone), 100 $\mu$ M non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol  $5.5 \times 10^{-5}$  M (Gibco), 10mM Hepes (Gibco) and IL-2 (1ng/ml). Following this, the activated Th1, Th2 and Tr1 lymphocytes were re-stimulated for 5 days with anti-CD28/OKT3 and cytokines as described above, but with the addition of anti-CD95L (1 $\mu$ g/ml) to prevent apoptosis. After 4-5 days, the Th1, Th2 and Tr1 lymphocytes were washed and then expanded again with IL-2 for 4-7 days. Activated Th1 and Th2 lymphocytes were maintained in this way for a maximum of three cycles. RNA was prepared from primary and secondary Th1, Th2 and Tr1 after 6 and 24 hours following the second and third activations with plate bound anti-CD3 and anti-CD28 mAbs and 4 days into the second and third expansion cultures in Interleukin 2.

The following leukocyte cells lines were obtained from the ATCC: Ramos, EOL-1, KU-812. EOL cells were further differentiated by culture in 0.1mM dbcAMP at  $5 \times 10^5$  cells/ml for 8 days, changing the media every 3 days and adjusting the cell concentration to  $5 \times 10^5$  cells/ml. For the culture of these cells, we used DMEM or RPMI (as recommended by the ATCC), with the addition of 5% FCS (Hyclone), 100 $\mu$ M non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol  $5.5 \times 10^{-5}$  M (Gibco), 10mM Hepes (Gibco). RNA was either prepared from resting cells or cells activated with PMA at 10ng/ml and ionomycin at 1 $\mu$ g/ml for 6 and 14 hours. Keratinocyte line CCD106 and an airway epithelial tumor line NCI-H292 were also obtained from the ATCC. Both were cultured in DMEM 5% FCS (Hyclone), 100 $\mu$ M non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol  $5.5 \times 10^{-5}$  M (Gibco), and 10mM Hepes (Gibco). CCD1106 cells were activated for 6 and 14 hours with approximately 5 ng/ml TNF alpha and 1ng/ml IL-1 beta, while NCI-H292 cells were activated for 6 and 14 hours with the following cytokines: 5ng/ml IL-4, 5ng/ml IL-9, 5ng/ml IL-13 and 25ng/ml IFN gamma.

For these cell lines and blood cells, RNA was prepared by lysing approximately  $10^7$  cells/ml using Trizol (Gibco BRL). Briefly, 1/10 volume of bromochloropropane (Molecular Research Corporation) was added to the RNA sample, vortexed and after 10 minutes at room temperature, the tubes were spun at 14,000 rpm in a Sorvall SS34 rotor. The

aqueous phase was removed and placed in a 15ml Falcon Tube. An equal volume of isopropanol was added and left at -20°C overnight. The precipitated RNA was spun down at 9,000 rpm for 15 min in a Sorvall SS34 rotor and washed in 70% ethanol. The pellet was redissolved in 300µl of RNase-free water and 35µl buffer (Promega) 5µl DTT, 7µl RNAsin and 8µl DNase were added. The tube was incubated at 37°C for 30 minutes to remove contaminating genomic DNA, extracted once with phenol chloroform and re-precipitated with 1/10 volume of 3M sodium acetate and 2 volumes of 100% ethanol. The RNA was spun down and placed in RNase free water. RNA was stored at -80°C.

#### AI\_comprehensive panel\_v1.0

The plates for AI\_comprehensive panel\_v1.0 include two control wells and 89 test samples comprised of cDNA isolated from surgical and postmortem human tissues obtained from the Backus Hospital and Clinomics (Frederick, MD). Total RNA was extracted from tissue samples from the Backus Hospital in the Facility at CuraGen. Total RNA from other tissues was obtained from Clinomics.

Joint tissues including synovial fluid, synovium, bone and cartilage were obtained from patients undergoing total knee or hip replacement surgery at the Backus Hospital. Tissue samples were immediately snap frozen in liquid nitrogen to ensure that isolated RNA was of optimal quality and not degraded. Additional samples of osteoarthritis and rheumatoid arthritis joint tissues were obtained from Clinomics. Normal control tissues were supplied by Clinomics and were obtained during autopsy of trauma victims.

Surgical specimens of psoriatic tissues and adjacent matched tissues were provided as total RNA by Clinomics. Two male and two female patients were selected between the ages of 25 and 47. None of the patients were taking prescription drugs at the time samples were isolated.

Surgical specimens of diseased colon from patients with ulcerative colitis and Crohns disease and adjacent matched tissues were obtained from Clinomics. Bowel tissue from three female and three male Crohn's patients between the ages of 41-69 were used. Two patients were not on prescription medication while the others were taking dexamethasone, phenobarbital, or tylenol. Ulcerative colitis tissue was from three male and four female patients. Four of the patients were taking lebid and two were on phenobarbital.

Total RNA from post mortem lung tissue from trauma victims with no disease or with emphysema, asthma or COPD was purchased from Clinomics. Emphysema patients ranged in age from 40-70 and all were smokers, this age range was chosen to focus on patients with

cigarette-linked emphysema and to avoid those patients with alpha-1 anti-trypsin deficiencies. Asthma patients ranged in age from 36-75, and excluded smokers to prevent those patients that could also have COPD. COPD patients ranged in age from 35-80 and included both smokers and non-smokers. Most patients were taking corticosteroids, and bronchodilators.

In the labels employed to identify tissues in the AI\_comprehensive panel\_v1.0 panel, the following abbreviations are used:

AI = Autoimmunity  
 Syn = Synovial  
 Normal = No apparent disease  
 Rep22 /Rep20 = individual patients  
 RA = Rheumatoid arthritis  
 Backus = From Backus Hospital  
 OA = Osteoarthritis  
 (SS) (BA) (MF) = Individual patients  
 Adj = Adjacent tissue  
 Match control = adjacent tissues  
 -M = Male  
 -F = Female  
 COPD = Chronic obstructive pulmonary disease

#### **Panels 5D and 5I**

The plates for Panel 5D and 5I include two control wells and a variety of cDNAs isolated from human tissues and cell lines with an emphasis on metabolic diseases. Metabolic tissues were obtained from patients enrolled in the Gestational Diabetes study. Cells were obtained during different stages in the differentiation of adipocytes from human mesenchymal stem cells. Human pancreatic islets were also obtained.

In the Gestational Diabetes study subjects are young (18 - 40 years), otherwise healthy women with and without gestational diabetes undergoing routine (elective) Caesarean section. After delivery of the infant, when the surgical incisions were being repaired/closed, the obstetrician removed a small sample.

Patient 2: Diabetic Hispanic, overweight, not on insulin  
 Patient 7-9: Nondiabetic Caucasian and obese (BMI>30)  
 Patient 10: Diabetic Hispanic, overweight, on insulin  
 Patient 11: Nondiabetic African American and overweight  
 Patient 12: Diabetic Hispanic on insulin

Adipocyte differentiation was induced in donor progenitor cells obtained from Osiris (a division of Clonetics/BioWhittaker) in triplicate, except for Donor 3U which had only two replicates. Scientists at Clonetics isolated, grew and differentiated human mesenchymal stem



cells (HuMSCs) for CuraGen based on the published protocol found in Mark F. Pittenger, et al., Multilineage Potential of Adult Human Mesenchymal Stem Cells Science Apr 2 1999: 143-147. Clonetics provided Trizol lysates or frozen pellets suitable for mRNA isolation and ds cDNA production. A general description of each donor is as follows:

Donor 2 and 3 U: Mesenchymal Stem cells, Undifferentiated Adipose  
Donor 2 and 3 AM: Adipose, AdiposeMidway Differentiated  
Donor 2 and 3 AD: Adipose, Adipose Differentiated

Human cell lines were generally obtained from ATCC (American Type Culture Collection), NCI or the German tumor cell bank and fall into the following tissue groups: kidney proximal convoluted tubule, uterine smooth muscle cells, small intestine, liver HepG2 cancer cells, heart primary stromal cells, and adrenal cortical adenoma cells. These cells are all cultured under standard recommended conditions and RNA extracted using the standard procedures. All samples were processed at CuraGen to produce single stranded cDNA.

Panel 5I contains all samples previously described with the addition of pancreatic islets from a 58 year old female patient obtained from the Diabetes Research Institute at the University of Miami School of Medicine. Islet tissue was processed to total RNA at an outside source and delivered to CuraGen for addition to panel 5I.

In the labels employed to identify tissues in the 5D and 5I panels, the following abbreviations are used:

GO Adipose = Greater Omentum Adipose  
SK = Skeletal Muscle  
UT = Uterus  
PL = Placenta  
AD = Adipose Differentiated  
AM = Adipose Midway Differentiated  
U = Undifferentiated Stem Cells

#### **Panel CNSD.01**

The plates for Panel CNSD.01 include two control wells and 94 test samples comprised of cDNA isolated from postmortem human brain tissue obtained from the Harvard Brain Tissue Resource Center. Brains are removed from calvaria of donors between 4 and 24 hours after death, sectioned by neuroanatomists, and frozen at -80°C in liquid nitrogen vapor. All brains are sectioned and examined by neuropathologists to confirm diagnoses with clear associated neuropathology.

Disease diagnoses are taken from patient records. The panel contains two brains from each of the following diagnoses: Alzheimer's disease, Parkinson's disease, Huntington's disease, Progressive Supranuclear Palsy, Depression, and "Normal controls". Within each of these brains, the following regions are represented: cingulate gyrus, temporal pole, globus palladus, substantia nigra, Brodman Area 4 (primary motor strip), Brodman Area 7 (parietal cortex), Brodman Area 9 (prefrontal cortex), and Brodman area 17 (occipital cortex). Not all brain regions are represented in all cases; e.g., Huntington's disease is characterized in part by neurodegeneration in the globus palladus, thus this region is impossible to obtain from confirmed Huntington's cases. Likewise Parkinson's disease is characterized by degeneration of the substantia nigra making this region more difficult to obtain. Normal control brains were examined for neuropathology and found to be free of any pathology consistent with neurodegeneration.

In the labels employed to identify tissues in the CNS panel, the following abbreviations are used:

PSP = Progressive supranuclear palsy  
 Sub Nigra = Substantia nigra  
 Glob Palladus = Globus palladus  
 Temp Pole = Temporal pole  
 Cing Gyr = Cingulate gyrus  
 BA 4 = Brodman Area 4

**Panel CNS\_Neurodegeneration\_V1.0**

The plates for Panel CNS\_Neurodegeneration\_V1.0 include two control wells and 47 test samples comprised of cDNA isolated from postmortem human brain tissue obtained from the Harvard Brain Tissue Resource Center (McLean Hospital) and the Human Brain and Spinal Fluid Resource Center (VA Greater Los Angeles Healthcare System). Brains are removed from calvaria of donors between 4 and 24 hours after death, sectioned by neuroanatomists, and frozen at -80°C in liquid nitrogen vapor. All brains are sectioned and examined by neuropathologists to confirm diagnoses with clear associated neuropathology.

Disease diagnoses are taken from patient records. The panel contains six brains from Alzheimer's disease (AD) patients, and eight brains from "Normal controls" who showed no evidence of dementia prior to death. The eight normal control brains are divided into two categories: Controls with no dementia and no Alzheimer's like pathology (Controls) and controls with no dementia but evidence of severe Alzheimer's like pathology, (specifically senile plaque load rated as level 3 on a scale of 0-3; 0 = no evidence of plaques, 3 = severe AD senile plaque load). Within each of these brains, the following regions are represented:

hippocampus, temporal cortex (Brodman Area 21), parietal cortex (Brodman area 7), and occipital cortex (Brodman area 17). These regions were chosen to encompass all levels of neurodegeneration in AD. The hippocampus is a region of early and severe neuronal loss in AD; the temporal cortex is known to show neurodegeneration in AD after the hippocampus; the parietal cortex shows moderate neuronal death in the late stages of the disease; the occipital cortex is spared in AD and therefore acts as a "control" region within AD patients. Not all brain regions are represented in all cases.

In the labels employed to identify tissues in the CNS\_Neurodegeneration\_V1.0 panel, the following abbreviations are used:

- AD = Alzheimer's disease brain; patient was demented and showed AD-like pathology upon autopsy  
 Control = Control brains; patient not demented, showing no neuropathology  
 Control (Path) = Control brains; patient not demented but showing severe AD-like pathology  
 SupTemporal Ctx = Superior Temporal Cortex  
 Inf Temporal Ctx = Inferior Temporal Cortex

#### NOV1b, NOV1c

- Expression of NOV1b and NOV1c was assessed using the primer-probe sets Ag1848, Ag2263, Ag2422 and Ag1522, described in Tables 14, 15, 16 and 17. Results of the RTQ-PCR runs are shown in Tables 18, 19, 20, 21, 22, 23 and 24.

**Table 14. Probe Name Ag1848**

| Primers | Sequences                                | Length | Start Position | SEQ ID NO: |
|---------|------------------------------------------|--------|----------------|------------|
| Forward | 5'-TGACTTCGACACAGACATCACT-3'             | 22     | 1234           | 133        |
| Probe   | TET-5'-ACTCATCTGCTGCCCTGACTGGTG-3'-TAMRA | 24     | 1257           | 134        |
| Reverse | 5'-CCTTGCCGTCTTAAAGTTGAC-3'              | 21     | 1292           | 135        |

**Table 15. Probe Name Ag2263**

| Primers | Sequences                                | Length | Start Position | SEQ ID NO: |
|---------|------------------------------------------|--------|----------------|------------|
| Forward | 5'-TGACTTCGACACAGACATCACT-3'             | 22     | 1234           | 136        |
| Probe   | TET-5'-ACTCATCTGCTGCCCTGACTGGTG-3'-TAMRA | 24     | 1257           | 137        |
| Reverse | 5'-CCTTGCCGTCTTAAAGTTGAC-3'              | 21     | 1292           | 138        |

**Table 16. Probe Name Ag2422**

| Primers | Sequences                 | Length | Start Position | SEQ ID NO: |
|---------|---------------------------|--------|----------------|------------|
| Forward | 5'-GGCTCCCTGGACACTCTCT-3' | 19     | 2522           | 139        |

|         |                                            |    |      |     |
|---------|--------------------------------------------|----|------|-----|
| Probe   | TET-5'-CTGTCACCACCCAGCTGGGACCTTAT-3'-TAMRA | 26 | 2559 | 140 |
| Reverse | 5'-TGGACAGTGGGATCTTGAAG-3'                 | 20 | 2587 | 141 |

Table 17. Probe Name Ag1522

| Primers | Sequences                                | Length | Start Position | SEQ ID NO: |
|---------|------------------------------------------|--------|----------------|------------|
| Forward | 5'-TGACTTCGACACAGACATCACT-3'             | 22     | 1234           | 142        |
| Probe   | TET-5'-ACTCATCTGCTGCCCTGACTGGTG-3'-TAMRA | 24     | 1257           | 143        |
| Reverse | 5'-CCTTGCCGTCTTAAAGTTGAC-3'              | 21     | 1292           | 144        |

Table 18. CNS\_neurodegeneration\_v1.0

| Tissue Name                   | Rel. Exp.(%)<br>Ag1848,<br>Run<br>207776125 | Rel. Exp.(%)<br>Ag2263,<br>Run<br>219933384 | Rel. Exp.(%)<br>Ag2263,<br>Run<br>224115886 | Rel. Exp.(%)<br>Ag2422,<br>Run<br>206262709 | Rel. Exp.(%)<br>Ag2422,<br>Run<br>230512499 |
|-------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| AD 1 Hippo                    | 28.3                                        | 39.0                                        | 19.3                                        | 21.3                                        | 16.6                                        |
| AD 2 Hippo                    | 37.9                                        | 45.1                                        | 23.5                                        | 38.7                                        | 40.1                                        |
| AD 3 Hippo                    | 12.0                                        | 20.6                                        | 13.9                                        | 14.9                                        | 13.0                                        |
| AD 4 Hippo                    | 17.7                                        | 27.2                                        | 9.0                                         | 13.3                                        | 16.4                                        |
| AD 5 Hippo                    | 45.4                                        | 60.3                                        | 8.1                                         | 57.8                                        | 59.0                                        |
| AD 6 Hippo                    | 66.9                                        | 96.6                                        | 70.2                                        | 95.9                                        | 66.0                                        |
| Control 2 Hippo               | 43.2                                        | 81.2                                        | 67.8                                        | 46.0                                        | 48.3                                        |
| Control 4 Hippo               | 34.2                                        | 36.6                                        | 38.7                                        | 30.4                                        | 27.5                                        |
| Control (Path) 3 Hippo        | 3.9                                         | 11.0                                        | 4.6                                         | 12.7                                        | 12.1                                        |
| AD 1 Temporal Ctx             | 47.0                                        | 79.0                                        | 69.7                                        | 40.6                                        | 27.2                                        |
| AD 2 Temporal Ctx             | 49.3                                        | 61.6                                        | 70.7                                        | 39.8                                        | 50.7                                        |
| AD 3 Temporal Ctx             | 14.5                                        | 20.7                                        | 15.3                                        | 15.7                                        | 14.5                                        |
| AD 4 Temporal Ctx             | 41.5                                        | 53.6                                        | 31.9                                        | 36.3                                        | 39.0                                        |
| AD 5 Inf Temporal Ctx         | 77.9                                        | 95.9                                        | 72.2                                        | 88.9                                        | 100.0                                       |
| AD 5 Sup Temporal Ctx         | 40.9                                        | 57.4                                        | 3.7                                         | 57.0                                        | 69.3                                        |
| AD 6 Inf Temporal Ctx         | 84.1                                        | 99.3                                        | 100.0                                       | 74.2                                        | 83.5                                        |
| AD 6 Sup Temporal Ctx         | 58.2                                        | 64.6                                        | 81.8                                        | 71.7                                        | 61.1                                        |
| Control 1 Temporal Ctx        | 17.9                                        | 18.0                                        | 21.5                                        | 11.3                                        | 16.5                                        |
| Control 2 Temporal Ctx        | 45.7                                        | 39.8                                        | 66.4                                        | 44.8                                        | 55.1                                        |
| Control 3 Temporal Ctx        | 14.7                                        | 21.8                                        | 22.7                                        | 15.6                                        | 13.5                                        |
| Control 3 Temporal Ctx        | 23.2                                        | 21.5                                        | 23.8                                        | 19.1                                        | 24.1                                        |
| Control (Path) 1 Temporal Ctx | 46.0                                        | 39.8                                        | 19.3                                        | 40.3                                        | 51.1                                        |
| Control (Path) 2 Temporal Ctx | 24.7                                        | 40.6                                        | 23.7                                        | 21.8                                        | 24.0                                        |
| Control (Path) 3 Temporal Ctx | 6.0                                         | 8.2                                         | 8.0                                         | 7.7                                         | 7.3                                         |

|                                |       |       |      |       |      |
|--------------------------------|-------|-------|------|-------|------|
| Control (Path) 4 Temporal Ctx  | 32.1  | 29.5  | 31.0 | 24.0  | 18.6 |
| AD 1 Occipital Ctx             | 24.1  | 48.0  | 5.5  | 26.4  | 13.7 |
| AD 2 Occipital Ctx (Missing)   | 0.0   | 0.0   | 0.0  | 0.0   | 0.0  |
| AD 3 Occipital Ctx             | 19.2  | 25.3  | 20.4 | 18.2  | 18.8 |
| AD 4 Occipital Ctx             | 30.1  | 58.2  | 30.6 | 23.3  | 30.8 |
| AD 5 Occipital Ctx             | 6.0   | 39.0  | 8.5  | 26.8  | 23.0 |
| AD 5 Occipital Ctx             | 43.2  | 51.8  | 53.6 | 50.3  | 47.6 |
| Control 1 Occipital Ctx        | 14.6  | 22.2  | 19.1 | 12.8  | 13.4 |
| Control 2 Occipital Ctx        | 66.9  | 85.9  | 94.6 | 76.3  | 70.2 |
| Control 3 Occipital Ctx        | 17.8  | 37.1  | 8.0  | 17.4  | 13.1 |
| Control 4 Occipital Ctx        | 23.3  | 22.2  | 2.7  | 15.7  | 19.1 |
| Control (Path) 1 Occipital Ctx | 100.0 | 100.0 | 63.7 | 100.0 | 90.1 |
| Control (Path) 2 Occipital Ctx | 18.7  | 20.9  | 11.0 | 12.3  | 11.7 |
| Control (Path) 3 Occipital Ctx | 7.9   | 6.1   | 9.4  | 7.1   | 5.8  |
| Control (Path) 4 Occipital Ctx | 24.5  | 21.5  | 11.1 | 14.0  | 13.1 |
| Control 1 Parietal Ctx         | 23.2  | 26.8  | 7.4  | 22.2  | 17.6 |
| Control 2 Parietal Ctx         | 46.0  | 65.1  | 71.2 | 64.6  | 50.0 |
| Control 3 Parietal Ctx         | 26.1  | 27.2  | 16.5 | 17.3  | 19.5 |
| Control (Path) 1 Parietal Ctx  | 51.1  | 66.0  | 80.1 | 54.3  | 55.1 |
| Control (Path) 2 Parietal Ctx  | 36.3  | 16.5  | 34.2 | 27.9  | 27.9 |
| Control (Path) 3 Parietal Ctx  | 6.1   | 10.5  | 1.4  | 5.1   | 4.6  |
| Control (Path) 4 Parietal Ctx  | 46.0  | 52.5  | 10.7 | 36.6  | 12.2 |

Table 19. Panel 1.2

| Tissue Name            | Rel. Exp.(%)<br>Ag1522, Run<br>142131145 | Tissue Name       | Rel. Exp.(%)<br>Ag1522, Run<br>142131145 |
|------------------------|------------------------------------------|-------------------|------------------------------------------|
| Endothelial cells      | 1.2                                      | Renal ca. 786-0   | 0.0                                      |
| Heart (Fetal)          | 17.9                                     | Renal ca. A498    | 0.3                                      |
| Pancreas               | 0.7                                      | Renal ca. RXF 393 | 0.2                                      |
| Pancreatic ca. CAPAN 2 | 4.9                                      | Renal ca. ACHN    | 0.1                                      |
| Adrenal Gland          | 7.9                                      | Renal ca. UO-31   | 0.5                                      |
| Thyroid                | 0.1                                      | Renal ca. TK-10   | 0.3                                      |
| Salivary gland         | 2.5                                      | Liver             | 2.4                                      |

|                              |       |                                |      |
|------------------------------|-------|--------------------------------|------|
| Pituitary gland              | 0.1   | Liver (fetal)                  | 0.5  |
| Brain (fetal)                | 0.2   | Liver ca. (hepatoblast) HepG2  | 0.3  |
| Brain (whole)                | 3.2   | Lung                           | 0.3  |
| Brain (amygdala)             | 4.4   | Lung (fetal)                   | 0.4  |
| Brain (cerebellum)           | 9.0   | Lung ca. (small cell) LX-1     | 25.3 |
| Brain (hippocampus)          | 18.9  | Lung ca. (small cell) NCI-H69  | 43.8 |
| Brain (thalamus)             | 15.7  | Lung ca. (s.cell var.) SHP-77  | 0.3  |
| Cerebral Cortex              | 35.4  | Lung ca. (large cell) NCI-H460 | 54.7 |
| Spinal cord                  | 1.6   | Lung ca. (non-sm. cell) A549   | 0.3  |
| glio/astro U87-MG            | 72.2  | Lung ca. (non-s.cell) NCI-H23  | 2.4  |
| glio/astro U-118-MG          | 3.1   | Lung ca. (non-s.cell) HOP-62   | 1.7  |
| astrocytoma SW1783           | 0.3   | Lung ca. (non-s.cl) NCI-H522   | 9.3  |
| neuro*; met SK-N-AS          | 36.3  | Lung ca. (squam.) SW 900       | 1.5  |
| astrocytoma SF-539           | 5.8   | Lung ca. (squam.) NCI-H596     | 22.4 |
| astrocytoma SNB-75           | 1.7   | Mammary gland                  | 1.4  |
| glioma SNB-19                | 23.8  | Breast ca.* (pl.ef) MCF-7      | 0.8  |
| glioma U251                  | 2.9   | Breast ca.* (pl.ef) MDA-MB-231 | 0.1  |
| glioma SF-295                | 100.0 | Breast ca.* (pl. ef) T47D      | 18.4 |
| Heart                        | 31.6  | Breast ca. BT-549              | 0.1  |
| Skeletal Muscle              | 3.4   | Breast ca. MDA-N               | 0.0  |
| Bone marrow                  | 0.2   | Ovary                          | 6.9  |
| Thymus                       | 0.2   | Ovarian ca. OVCAR-3            | 1.7  |
| Spleen                       | 2.1   | Ovarian ca. OVCAR-4            | 12.9 |
| Lymph node                   | 0.5   | Ovarian ca. OVCAR-5            | 5.7  |
| Colorectal                   | 1.4   | Ovarian ca. OVCAR-8            | 5.3  |
| Stomach                      | 1.3   | Ovarian ca. IGROV-1            | 0.8  |
| Small intestine              | 3.3   | Ovarian ca. (ascites) SK-OV-3  | 5.4  |
| Colon ca. SW480              | 0.8   | Uterus                         | 0.9  |
| Colon ca.* SW620 (SW480 met) | 2.2   | Placenta                       | 0.9  |
| Colon ca. HT29               | 0.1   | Prostate                       | 10.0 |
| Colon ca. HCT-116            | 7.5   | Prostate ca.* (bone met) PC-3  | 0.1  |

|                                     |      |                               |      |
|-------------------------------------|------|-------------------------------|------|
| Colon ca. CaCo-2                    | 6.3  | Testis                        | 0.3  |
| CC Well to Mod Diff<br>(ODO3866)    | 3.0  | Melanoma Hs688(A).T           | 21.2 |
| Colon ca. HCC-2998                  | 1.2  | Melanoma* (met)<br>Hs688(B).T | 28.5 |
| Gastric ca. (liver met) NCI-<br>N87 | 24.7 | Melanoma UACC-62              | 2.4  |
| Bladder                             | 12.8 | Melanoma M14                  | 0.1  |
| Trachea                             | 0.3  | Melanoma LOX IMVI             | 0.1  |
| Kidney                              | 19.2 | Melanoma* (met) SK-<br>MEL-5  | 1.2  |
| Kidney (fetal)                      | 6.6  |                               |      |

Table 20. Panel 1.3D

| Tissue Name              | Rel. Exp.(%)<br>Ag1522, Run<br>159601761 | Rel. Exp.(%)<br>Ag1848, Run<br>160201402 | Rel. Exp.(%)<br>Ag2263, Run<br>166011650 | Rel. Exp.(%)<br>Ag2422, Run<br>159319549 |
|--------------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|
| Liver adenocarcinoma     | 15.8                                     | 12.3                                     | 31.4                                     | 18.3                                     |
| Pancreas                 | 1.7                                      | 1.4                                      | 2.8                                      | 2.9                                      |
| Pancreatic ca. CAPAN 2   | 6.7                                      | 4.6                                      | 21.6                                     | 5.5                                      |
| Adrenal gland            | 3.9                                      | 2.0                                      | 3.5                                      | 3.0                                      |
| Thyroid                  | 1.7                                      | 1.5                                      | 0.0                                      | 2.5                                      |
| Salivary gland           | 0.6                                      | 0.2                                      | 2.3                                      | 0.3                                      |
| Pituitary gland          | 2.1                                      | 1.4                                      | 2.9                                      | 4.3                                      |
| Brain (fetal)            | 1.4                                      | 1.1                                      | 3.5                                      | 1.1                                      |
| Brain (whole)            | 28.7                                     | 13.5                                     | 43.2                                     | 10.4                                     |
| Brain (amygdala)         | 16.8                                     | 13.0                                     | 31.2                                     | 18.6                                     |
| Brain (cerebellum)       | 8.2                                      | 6.5                                      | 42.3                                     | 9.2                                      |
| Brain (hippocampus)      | 60.7                                     | 47.6                                     | 16.8                                     | 51.8                                     |
| Brain (substantia nigra) | 8.9                                      | 5.2                                      | 32.3                                     | 6.8                                      |
| Brain (thalamus)         | 40.1                                     | 22.2                                     | 62.0                                     | 19.8                                     |
| Cerebral Cortex          | 25.9                                     | 18.4                                     | 36.6                                     | 14.3                                     |
| Spinal cord              | 10.2                                     | 5.4                                      | 37.9                                     | 7.9                                      |
| glio/astro U87-MG        | 43.2                                     | 34.6                                     | 100.0                                    | 48.6                                     |
| glio/astro U-118-MG      | 10.2                                     | 8.0                                      | 6.4                                      | 7.5                                      |
| astrocytoma SW1783       | 0.9                                      | 0.8                                      | 2.8                                      | 1.1                                      |
| neuro*; met SK-N-AS      | 100.0                                    | 100.0                                    | 59.0                                     | 100.0                                    |
| astrocytoma SF-539       | 9.7                                      | 8.3                                      | 17.7                                     | 9.0                                      |
| astrocytoma SNB-75       | 12.9                                     | 12.1                                     | 8.4                                      | 12.1                                     |
| glioma SNB-19            | 19.5                                     | 17.6                                     | 46.3                                     | 17.2                                     |
| glioma U251              | 13.4                                     | 10.6                                     | 24.5                                     | 10.9                                     |
| glioma SF-295            | 66.9                                     | 62.4                                     | 64.2                                     | 62.0                                     |
| Heart (Fetal)            | 15.6                                     | 12.5                                     | 20.0                                     | 18.7                                     |

|                                  |      |      |      |      |
|----------------------------------|------|------|------|------|
| Heart                            | 2.2  | 1.1  | 3.4  | 3.3  |
| Skeletal muscle (Fetal)          | 22.2 | 14.0 | 6.7  | 19.3 |
| Skeletal muscle                  | 0.3  | 0.2  | 1.4  | 0.7  |
| Bone marrow                      | 0.7  | 0.3  | 0.4  | 0.8  |
| Thymus                           | 2.0  | 1.6  | 3.6  | 3.4  |
| Spleen                           | 7.9  | 5.6  | 4.5  | 5.9  |
| Lymph node                       | 2.6  | 1.9  | 2.7  | 2.1  |
| Colorectal                       | 4.7  | 9.2  | 12.8 | 10.3 |
| Stomach                          | 6.1  | 2.4  | 3.6  | 4.5  |
| Small intestine                  | 2.9  | 2.9  | 4.5  | 4.9  |
| Colon ca. SW480                  | 2.0  | 1.0  | 1.9  | 1.5  |
| Colon ca.* SW620 (SW480 met)     | 1.0  | 1.2  | 2.0  | 2.1  |
| Colon ca. HT29                   | 0.1  | 0.1  | 0.0  | 0.1  |
| Colon ca. HCT-116                | 4.2  | 2.9  | 4.7  | 5.6  |
| Colon ca. CaCo-2                 | 5.3  | 3.9  | 12.5 | 7.2  |
| CC Well to Mod Diff<br>(ODO3866) | 14.8 | 17.3 | 19.8 | 23.5 |
| Colon ca. HCC-2998               | 0.7  | 1.6  | 0.0  | 0.5  |
| Gastric ca. (liver met) NCI-N87  | 21.9 | 22.8 | 19.1 | 25.7 |
| Bladder                          | 2.1  | 1.7  | 3.4  | 1.5  |
| Trachea                          | 12.2 | 6.8  | 1.6  | 13.8 |
| Kidney                           | 1.4  | 0.6  | 3.9  | 3.0  |
| Kidney (fetal)                   | 5.3  | 5.8  | 5.2  | 6.3  |
| Renal ca. 786-0                  | 0.1  | 0.0  | 0.0  | 0.0  |
| Renal ca. A498                   | 7.7  | 7.9  | 6.8  | 9.7  |
| Renal ca. RXF 393                | 0.1  | 3.6  | 0.8  | 0.1  |
| Renal ca. ACHN                   | 0.0  | 0.0  | 0.0  | 0.0  |
| Renal ca. UO-31                  | 0.2  | 0.3  | 0.5  | 0.3  |
| Renal ca. TK-10                  | 0.1  | 0.0  | 0.0  | 0.0  |
| Liver                            | 0.3  | 0.1  | 0.0  | 0.6  |
| Liver (fetal)                    | 1.1  | 1.0  | 0.3  | 1.2  |
| Liver ca. (hepatoblast) HepG2    | 0.2  | 0.0  | 0.8  | 0.3  |
| Lung                             | 8.2  | 9.4  | 4.1  | 10.3 |
| Lung (fetal)                     | 4.3  | 4.2  | 7.3  | 4.5  |
| Lung ca. (small cell) LX-1       | 8.4  | 6.9  | 31.6 | 9.9  |
| Lung ca. (small cell) NCI-H69    | 44.4 | 48.6 | 90.8 | 54.3 |
| Lung ca. (s.cell var.) SHP-77    | 0.7  | 0.8  | 0.5  | 1.1  |
| Lung ca. (large cell) NCI-H460   | 16.2 | 11.9 | 22.4 | 12.1 |
| Lung ca. (non-sm. cell) A549     | 0.4  | 0.3  | 0.2  | 0.4  |
| Lung ca. (non-s.cell) NCI-H23    | 2.0  | 0.9  | 3.3  | 1.2  |
| Lung ca. (non-s.cell) HOP-62     | 0.4  | 0.9  | 1.6  | 0.7  |
| Lung ca. (non-s.cl) NCI-H522     | 1.7  | 0.8  | 1.7  | 1.1  |



|                                |      |      |      |      |
|--------------------------------|------|------|------|------|
| Lung ca. (squam.) SW 900       | 0.5  | 0.3  | 1.9  | 0.2  |
| Lung ca. (squam.) NCI-H596     | 4.0  | 4.1  | 26.4 | 2.4  |
| Mammary gland                  | 6.3  | 4.4  | 3.0  | 2.8  |
| Breast ca.* (pl.ef) MCF-7      | 1.1  | 0.4  | 1.5  | 0.9  |
| Breast ca.* (pl.ef) MDA-MB-231 | 0.8  | 1.2  | 0.7  | 1.4  |
| Breast ca.* (pl. ef) T47D      | 9.6  | 5.7  | 14.0 | 4.5  |
| Breast ca. BT-549              | 0.2  | 0.3  | 0.2  | 0.3  |
| Breast ca. MDA-N               | 0.0  | 0.0  | 0.0  | 0.0  |
| Ovary                          | 6.4  | 4.9  | 6.2  | 9.5  |
| Ovarian ca. OVCAR-3            | 1.1  | 0.6  | 1.1  | 0.8  |
| Ovarian ca. OVCAR-4            | 1.0  | 1.4  | 11.4 | 1.5  |
| Ovarian ca. OVCAR-5            | 2.4  | 2.6  | 5.7  | 3.3  |
| Ovarian ca. OVCAR-8            | 3.6  | 1.6  | 2.6  | 5.4  |
| Ovarian ca. IGROV-1            | 0.6  | 0.2  | 0.7  | 0.2  |
| Ovarian ca. (ascites) SK-OV-3  | 2.0  | 2.6  | 2.1  | 1.1  |
| Uterus                         | 2.7  | 1.3  | 3.9  | 4.2  |
| Placenta                       | 2.0  | 2.0  | 5.8  | 4.8  |
| Prostate                       | 4.4  | 2.5  | 3.4  | 5.4  |
| Prostate ca.* (bone met) PC-3  | 0.1  | 0.1  | 0.2  | 0.0  |
| Testis                         | 8.1  | 5.5  | 3.5  | 6.4  |
| Melanoma Hs688(A).T            | 31.6 | 25.0 | 59.5 | 27.4 |
| Melanoma* (met) Hs688(B).T     | 46.0 | 17.1 | 87.1 | 28.5 |
| Melanoma UACC-62               | 0.1  | 0.2  | 2.0  | 0.5  |
| Melanoma M14                   | 0.0  | 0.0  | 0.0  | 0.0  |
| Melanoma LOX IMVI              | 0.1  | 0.2  | 0.0  | 0.1  |
| Melanoma* (met) SK-MEL-5       | 0.9  | 0.9  | 1.7  | 0.6  |
| Adipose                        | 3.6  | 2.3  | 5.1  | 2.9  |

Table 21. Panel 2D

| Tissue Name                          | Rel. Exp.(%)<br>Ag1522, Run<br>145049854 | Rel. Exp.(%)<br>Ag1522, Run<br>145492337 | Rel. Exp.(%)<br>Ag1848, Run<br>160202834 | Rel. Exp.(%)<br>Ag2263, Run<br>165725935 | Rel. Exp.(%)<br>Ag2422, Run<br>159317774 |
|--------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|
| Normal Colon                         | 20.2                                     | 46.0                                     | 35.1                                     | 59.0                                     | 36.9                                     |
| CC Well to Mod<br>Diff (ODO3866)     | 15.3                                     | 45.1                                     | 22.5                                     | 21.8                                     | 21.3                                     |
| CC Margin<br>(ODO3866)               | 6.1                                      | 15.2                                     | 7.4                                      | 7.7                                      | 5.5                                      |
| CC Gr.2<br>rectosigmoid<br>(ODO3868) | 7.0                                      | 8.2                                      | 5.8                                      | 5.9                                      | 13.2                                     |
| CC Margin<br>(ODO3868)               | 0.3                                      | 0.5                                      | 0.5                                      | 9.3                                      | 0.8                                      |
| CC Mod Diff                          | 1.2                                      | 4.0                                      | 2.5                                      | 5.6                                      | 5.8                                      |

|                                                     |      |      |      |      |      |
|-----------------------------------------------------|------|------|------|------|------|
| (ODO3920)                                           |      |      |      |      |      |
| CC Margin<br>(ODO3920)                              | 3.0  | 4.7  | 4.1  | 5.4  | 7.2  |
| CC Gr.2 ascend<br>colon<br>(ODO3921)                | 10.7 | 22.5 | 24.1 | 19.9 | 25.5 |
| CC Margin<br>(ODO3921)                              | 3.6  | 4.3  | 7.3  | 5.6  | 5.8  |
| CC from Partial<br>Hepatectomy<br>(ODO4309)<br>Mets | 12.1 | 19.9 | 20.7 | 19.3 | 27.0 |
| Liver Margin<br>(ODO4309)                           | 0.4  | 3.6  | 2.4  | 2.6  | 3.3  |
| Colon mets to<br>lung (OD04451-<br>01)              | 5.8  | 11.9 | 6.1  | 8.5  | 10.7 |
| Lung Margin<br>(OD04451-02)                         | 9.3  | 17.7 | 7.7  | 10.0 | 15.4 |
| Normal Prostate<br>6546-1                           | 10.5 | 51.1 | 7.3  | 21.6 | 7.0  |
| Prostate Cancer<br>(OD04410)                        | 12.2 | 14.9 | 14.9 | 9.0  | 17.4 |
| Prostate Margin<br>(OD04410)                        | 14.6 | 13.8 | 25.3 | 19.2 | 29.7 |
| Prostate Cancer<br>(OD04720-01)                     | 12.2 | 18.0 | 22.7 | 31.6 | 30.6 |
| Prostate Margin<br>(OD04720-02)                     | 11.8 | 11.8 | 17.7 | 16.7 | 25.0 |
| Normal Lung                                         | 7.3  | 17.8 | 17.6 | 12.8 | 22.4 |
| Lung Met to<br>Muscle<br>(ODO4286)                  | 12.7 | 27.4 | 25.0 | 31.0 | 22.1 |
| Muscle Margin<br>(ODO4286)                          | 7.4  | 8.7  | 6.2  | 7.3  | 9.5  |
| Lung Malignant<br>Cancer<br>(OD03126)               | 22.7 | 27.4 | 26.1 | 28.3 | 20.4 |
| Lung Margin<br>(OD03126)                            | 12.7 | 21.9 | 21.9 | 13.9 | 31.9 |
| Lung Cancer<br>(OD04404)                            | 17.9 | 41.5 | 41.5 | 30.4 | 48.0 |
| Lung Margin<br>(OD04404)                            | 16.4 | 28.7 | 10.0 | 11.8 | 12.4 |
| Lung Cancer<br>(OD04565)                            | 22.5 | 38.2 | 28.5 | 27.9 | 40.6 |

|                                       |      |      |      |      |      |
|---------------------------------------|------|------|------|------|------|
| Lung Margin (OD04565)                 | 8.1  | 11.7 | 8.5  | 8.6  | 16.3 |
| Lung Cancer (OD04237-01)              | 9.8  | 7.1  | 10.9 | 8.8  | 9.6  |
| Lung Margin (OD04237-02)              | 12.9 | 23.0 | 14.3 | 14.0 | 16.0 |
| Ocular Mel Met to Liver (ODO4310)     | 0.6  | 0.5  | 0.7  | 0.5  | 1.1  |
| Liver Margin (ODO4310)                | 3.5  | 2.6  | 1.8  | 3.3  | 3.0  |
| Melanoma Metastasis                   | 1.4  | 2.0  | 3.6  | 4.3  | 2.9  |
| Lung Margin (OD04321)                 | 20.4 | 14.4 | 25.2 | 24.0 | 18.6 |
| Normal Kidney                         | 20.2 | 19.9 | 18.0 | 17.4 | 26.1 |
| Kidney Ca, Nuclear grade 2 (OD04338)  | 1.7  | 4.2  | 2.9  | 2.7  | 4.9  |
| Kidney Margin (OD04338)               | 6.2  | 11.7 | 17.2 | 11.3 | 22.8 |
| Kidney Ca Nuclear grade 1/2 (OD04339) | 3.6  | 10.0 | 3.7  | 4.6  | 6.6  |
| Kidney Margin (OD04339)               | 11.7 | 12.2 | 11.4 | 12.1 | 11.0 |
| Kidney Ca, Clear cell type (OD04340)  | 46.7 | 50.7 | 66.0 | 65.1 | 70.7 |
| Kidney Margin (OD04340)               | 15.3 | 19.1 | 14.8 | 12.9 | 16.8 |
| Kidney Ca, Nuclear grade 3 (OD04348)  | 21.0 | 9.5  | 16.3 | 16.8 | 17.0 |
| Kidney Margin (OD04348)               | 8.2  | 5.8  | 8.8  | 11.5 | 9.3  |
| Kidney Cancer (OD04622-01)            | 24.0 | 25.3 | 27.7 | 24.8 | 41.5 |
| Kidney Margin (OD04622-03)            | 2.1  | 4.6  | 3.4  | 3.1  | 5.9  |
| Kidney Cancer (OD04450-01)            | 0.2  | 0.0  | 0.2  | 0.5  | 0.5  |
| Kidney Margin (OD04450-03)            | 5.9  | 6.3  | 9.3  | 9.9  | 12.9 |
| Kidney Cancer 8120607                 | 7.3  | 9.1  | 11.9 | 12.8 | 13.4 |

|                                    |       |      |       |      |       |
|------------------------------------|-------|------|-------|------|-------|
| Kidney Margin<br>8120608           | 12.2  | 6.2  | 7.9   | 5.6  | 8.0   |
| Kidney Cancer<br>8120613           | 3.6   | 8.0  | 5.2   | 8.8  | 10.1  |
| Kidney Margin<br>8120614           | 6.3   | 6.7  | 8.9   | 7.5  | 9.3   |
| Kidney Cancer<br>9010320           | 18.7  | 61.1 | 25.0  | 21.9 | 22.1  |
| Kidney Margin<br>9010321           | 14.0  | 20.3 | 16.4  | 12.9 | 17.9  |
| Normal Uterus                      | 4.1   | 5.6  | 3.3   | 8.4  | 6.0   |
| Uterine Cancer<br>064011           | 9.6   | 10.7 | 17.1  | 11.7 | 15.6  |
| Normal Thyroid                     | 2.6   | 9.2  | 2.6   | 1.5  | 3.6   |
| Thyroid Cancer                     | 100.0 | 72.7 | 100.0 | 82.9 | 100.0 |
| Thyroid Cancer<br>A302152          | 7.6   | 4.5  | 12.5  | 8.0  | 11.7  |
| Thyroid Margin<br>A302153          | 3.0   | 2.4  | 2.8   | 3.2  | 6.0   |
| Normal Breast                      | 10.3  | 5.7  | 9.9   | 12.9 | 7.2   |
| Breast Cancer                      | 11.7  | 15.9 | 12.8  | 12.9 | 12.8  |
| Breast Cancer<br>(OD04590-01)      | 17.9  | 39.0 | 27.2  | 16.5 | 25.3  |
| Breast Cancer<br>Mets (OD04590-03) | 26.1  | 66.0 | 35.4  | 42.0 | 27.9  |
| Breast Cancer<br>Metastasis        | 4.5   | 5.4  | 6.0   | 5.2  | 3.5   |
| Breast Cancer                      | 30.8  | 32.1 | 28.1  | 21.6 | 36.3  |
| Breast Cancer                      | 20.7  | 46.7 | 19.8  | 16.7 | 14.8  |
| Breast Cancer<br>9100266           | 13.1  | 15.9 | 13.9  | 11.0 | 22.1  |
| Breast Margin<br>9100265           | 10.4  | 14.4 | 15.6  | 16.4 | 20.9  |
| Breast Cancer<br>A209073           | 22.2  | 26.8 | 34.2  | 25.5 | 50.0  |
| Breast Margin<br>A2090734          | 6.7   | 9.7  | 7.1   | 4.3  | 11.3  |
| Normal Liver                       | 1.4   | 4.2  | 1.6   | 1.7  | 2.3   |
| Liver Cancer                       | 1.0   | 2.8  | 1.7   | 1.3  | 1.3   |
| Liver Cancer<br>1025               | 1.4   | 1.1  | 3.3   | 2.3  | 3.2   |
| Liver Cancer<br>1026               | 7.8   | 6.5  | 4.9   | 6.4  | 10.7  |
| Liver Cancer                       | 5.0   | 9.9  | 4.2   | 3.0  | 5.2   |

|                                            |      |       |      |       |      |
|--------------------------------------------|------|-------|------|-------|------|
| 6004-T                                     |      |       |      |       |      |
| Liver Tissue<br>6004-N                     | 4.7  | 7.9   | 3.5  | 4.2   | 3.7  |
| Liver Cancer<br>6005-T                     | 7.9  | 11.5  | 8.2  | 10.3  | 6.7  |
| Liver Tissue<br>6005-N                     | 2.0  | 3.2   | 2.7  | 1.6   | 2.3  |
| Normal Bladder                             | 6.8  | 17.9  | 13.6 | 11.5  | 15.2 |
| Bladder Cancer                             | 10.7 | 22.8  | 14.5 | 14.2  | 14.2 |
| Bladder Cancer                             | 18.0 | 29.3  | 22.7 | 17.7  | 23.5 |
| Bladder Cancer<br>(OD04718-01)             | 14.5 | 29.3  | 26.1 | 21.0  | 28.3 |
| Bladder Normal<br>Adjacent<br>(OD04718-03) | 2.9  | 5.0   | 3.1  | 3.2   | 4.2  |
| Normal Ovary                               | 1.4  | 4.7   | 3.6  | 4.6   | 5.4  |
| Ovarian Cancer                             | 40.9 | 100.0 | 89.5 | 100.0 | 76.3 |
| Ovarian Cancer<br>(OD04768-07)             | 9.7  | 43.2  | 16.7 | 15.6  | 19.5 |
| Ovary Margin<br>(OD04768-08)               | 6.5  | 7.9   | 10.8 | 6.7   | 8.3  |
| Normal Stomach                             | 11.8 | 39.5  | 14.7 | 14.8  | 13.1 |
| Gastric Cancer<br>9060358                  | 1.4  | 6.0   | 2.9  | 2.8   | 2.9  |
| Stomach Margin<br>9060359                  | 6.4  | 19.9  | 7.4  | 10.8  | 8.7  |
| Gastric Cancer<br>9060395                  | 11.1 | 58.6  | 21.6 | 21.2  | 32.3 |
| Stomach Margin<br>9060394                  | 6.8  | 34.6  | 23.7 | 13.8  | 22.2 |
| Gastric Cancer<br>9060397                  | 15.4 | 78.5  | 24.8 | 25.2  | 31.9 |
| Stomach Margin<br>9060396                  | 3.9  | 14.5  | 6.1  | 7.5   | 7.9  |
| Gastric Cancer<br>064005                   | 2.5  | 14.8  | 7.0  | 7.3   | 13.0 |

Table 22. Panel 3D

| Tissue Name            | Rel. Exp.(%)<br>Ag2263, Run<br>170189128 | Tissue Name                                           | Rel. Exp.(%)<br>Ag2263, Run<br>170189128 |
|------------------------|------------------------------------------|-------------------------------------------------------|------------------------------------------|
| Daoy- Medulloblastoma  | 19.1                                     | Ca Ski- Cervical epidermoid<br>carcinoma (metastasis) | 0.4                                      |
| TE671- Medulloblastoma | 8.4                                      | ES-2- Ovarian clear cell<br>carcinoma                 | 0.0                                      |
| D283 Med-              | 39.2                                     | Ramos- Stimulated with                                | 0.0                                      |

|                                                  |       |                                                       |      |
|--------------------------------------------------|-------|-------------------------------------------------------|------|
| Medulloblastoma                                  |       | PMA/ionomycin 6h                                      |      |
| PFSK-1- Primitive Neuroectodermal                | 59.5  | Ramos- Stimulated with PMA/ionomycin 14h              | 0.0  |
| XF-498- CNS                                      | 0.9   | MEG-01- Chronic myelogenous leukemia (megokaryoblast) | 3.8  |
| SNB-78- Glioma                                   | 35.4  | Raji- Burkitt's lymphoma                              | 0.0  |
| SF-268- Glioblastoma                             | 0.0   | Daudi- Burkitt's lymphoma                             | 0.0  |
| T98G- Glioblastoma                               | 1.2   | U266- B-cell plasmacytoma                             | 0.0  |
| SK-N-SH- Neuroblastoma (metastasis)              | 94.6  | CA46- Burkitt's lymphoma                              | 0.0  |
| SF-295- Glioblastoma                             | 0.3   | RL- non-Hodgkin's B-cell lymphoma                     | 0.0  |
| Cerebellum                                       | 37.4  | JM1- pre-B-cell lymphoma                              | 0.0  |
| Cerebellum                                       | 35.1  | Jurkat- T cell leukemia                               | 0.5  |
| NCI-H292- Mucoepidermoid lung carcinoma          | 4.3   | TF-1- Erythroleukemia                                 | 73.2 |
| DMS-114- Small cell lung cancer                  | 6.6   | HUT 78- T-cell lymphoma                               | 0.0  |
| DMS-79- Small cell lung cancer                   | 100.0 | U937- Histiocytic lymphoma                            | 0.0  |
| NCI-H146- Small cell lung cancer                 | 37.4  | KU-812- Myelogenous leukemia                          | 0.6  |
| NCI-H526- Small cell lung cancer                 | 17.2  | 769-P- Clear cell renal carcinoma                     | 0.0  |
| NCI-N417- Small cell lung cancer                 | 88.9  | Caki-2- Clear cell renal carcinoma                    | 0.0  |
| NCI-H82- Small cell lung cancer                  | 95.3  | SW 839- Clear cell renal carcinoma                    | 0.0  |
| NCI-H157- Squamous cell lung cancer (metastasis) | 0.8   | G401- Wilms' tumor                                    | 2.8  |
| NCI-H1155- Large cell lung cancer                | 55.5  | Hs766T- Pancreatic carcinoma (LN metastasis)          | 0.6  |
| NCI-H1299- Large cell lung cancer                | 0.0   | CAPAN-1- Pancreatic adenocarcinoma (liver metastasis) | 3.1  |
| NCI-H727- Lung carcinoid                         | 0.7   | SU86.86- Pancreatic carcinoma (liver metastasis)      | 0.4  |
| NCI-UMC-11- Lung carcinoid                       | 7.9   | BxPC-3- Pancreatic adenocarcinoma                     | 22.8 |
| LX-1- Small cell lung cancer                     | 1.8   | HPAC- Pancreatic adenocarcinoma                       | 35.6 |
| Colo-205- Colon cancer                           | 0.3   | MIA PaCa-2- Pancreatic                                | 0.6  |

|                                 |      |                                                 |      |
|---------------------------------|------|-------------------------------------------------|------|
|                                 |      | carcinoma                                       |      |
| KM12- Colon cancer              | 0.1  | CFPAC-1- Pancreatic ductal adenocarcinoma       | 1.1  |
| KM20L2- Colon cancer            | 0.6  | PANC-1- Pancreatic epithelioid ductal carcinoma | 0.3  |
| NCI-H716- Colon cancer          | 70.2 | T24- Bladder carcinoma (transitional cell)      | 0.0  |
| SW-48- Colon adenocarcinoma     | 0.0  | 5637- Bladder carcinoma                         | 2.2  |
| SW1116- Colon adenocarcinoma    | 16.6 | HT-1197- Bladder carcinoma                      | 0.4  |
| LS 174T- Colon adenocarcinoma   | 4.2  | UM-UC-3- Bladder carcinoma (transitional cell)  | 0.2  |
| SW-948- Colon adenocarcinoma    | 0.4  | A204- Rhabdomyosarcoma                          | 0.0  |
| SW-480- Colon adenocarcinoma    | 0.0  | HT-1080- Fibrosarcoma                           | 7.9  |
| NCI-SNU-5- Gastric carcinoma    | 1.7  | MG-63- Osteosarcoma                             | 16.3 |
| KATO III- Gastric carcinoma     | 17.4 | SK-LMS-1- Leiomyosarcoma (vulva)                | 0.0  |
| NCI-SNU-16- Gastric carcinoma   | 0.7  | SJRH30- Rhabdomyosarcoma (met to bone marrow)   | 3.9  |
| NCI-SNU-1- Gastric carcinoma    | 23.0 | A431- Epidermoid carcinoma                      | 34.9 |
| RF-1- Gastric adenocarcinoma    | 0.0  | WM266-4- Melanoma                               | 0.0  |
| RF-48- Gastric adenocarcinoma   | 0.0  | DU 145- Prostate carcinoma (brain metastasis)   | 0.0  |
| MKN-45- Gastric carcinoma       | 11.5 | MDA-MB-468- Breast adenocarcinoma               | 16.4 |
| NCI-N87- Gastric carcinoma      | 24.0 | SCC-4- Squamous cell carcinoma of tongue        | 0.0  |
| OVCAR-5- Ovarian carcinoma      | 3.7  | SCC-9- Squamous cell carcinoma of tongue        | 0.0  |
| RL95-2- Uterine carcinoma       | 4.6  | SCC-15- Squamous cell carcinoma of tongue       | 0.0  |
| HelaS3- Cervical adenocarcinoma | 5.9  | CAL 27- Squamous cell carcinoma of tongue       | 7.1  |

Table 23. Panel 4D

| Tissue Name       | Rel. Exp.(%)<br>Ag1522, Run<br>145789191 | Rel. Exp.(%)<br>Ag1848, Run<br>160202841 | Rel. Exp.(%)<br>Ag2263, Run<br>151562852 | Rel. Exp.(%)<br>Ag2422, Run<br>159318890 |
|-------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|
| Secondary Th1 act | 0.0                                      | 0.1                                      | 0.0                                      | 0.2                                      |
| Secondary Th2 act | 0.0                                      | 0.0                                      | 0.0                                      | 0.0                                      |

|                                |     |     |     |      |
|--------------------------------|-----|-----|-----|------|
| Secondary Tr1 act              | 0.0 | 0.0 | 0.0 | 4.6  |
| Secondary Th1 rest             | 0.1 | 0.0 | 0.1 | 0.0  |
| Secondary Th2 rest             | 0.0 | 0.0 | 0.0 | 0.0  |
| Secondary Tr1 rest             | 0.0 | 0.0 | 0.0 | 0.2  |
| Primary Th1 act                | 0.1 | 0.2 | 0.2 | 1.0  |
| Primary Th2 act                | 0.1 | 0.2 | 0.1 | 0.3  |
| Primary Tr1 act                | 0.2 | 0.5 | 0.0 | 0.6  |
| Primary Th1 rest               | 0.0 | 0.0 | 0.0 | 0.0  |
| Primary Th2 rest               | 0.0 | 0.0 | 0.0 | 0.0  |
| Primary Tr1 rest               | 0.0 | 0.0 | 0.0 | 0.0  |
| CD45RA CD4 lymphocyte act      | 4.9 | 6.3 | 8.5 | 10.6 |
| CD45RO CD4 lymphocyte act      | 0.0 | 0.0 | 0.0 | 0.0  |
| CD8 lymphocyte act             | 0.0 | 0.0 | 0.0 | 0.0  |
| Secondary CD8 lymphocyte rest  | 0.0 | 0.0 | 0.0 | 0.0  |
| Secondary CD8 lymphocyte act   | 0.0 | 0.0 | 0.0 | 0.0  |
| CD4 lymphocyte none            | 0.0 | 0.0 | 0.0 | 0.0  |
| 2ry Th1/Th2/Tr1_anti-CD95 CH11 | 0.0 | 0.0 | 0.0 | 0.0  |
| LAK cells rest                 | 1.8 | 2.7 | 2.0 | 5.8  |
| LAK cells IL-2                 | 0.0 | 0.0 | 0.0 | 0.0  |
| LAK cells IL-2+IL-12           | 0.0 | 0.1 | 0.0 | 0.2  |
| LAK cells IL-2+IFN gamma       | 0.0 | 0.1 | 0.0 | 0.2  |
| LAK cells IL-2+ IL-18          | 0.0 | 0.4 | 0.0 | 0.1  |
| LAK cells PMA/ionomycin        | 1.1 | 1.0 | 1.7 | 2.5  |
| NK Cells IL-2 rest             | 0.0 | 0.1 | 0.0 | 0.0  |
| Two Way MLR 3 day              | 0.0 | 0.1 | 0.2 | 0.2  |
| Two Way MLR 5 day              | 0.2 | 0.3 | 0.8 | 0.6  |
| Two Way MLR 7 day              | 0.5 | 0.2 | 0.1 | 0.3  |
| PBMC rest                      | 0.0 | 0.0 | 0.1 | 0.0  |
| PBMC PWM                       | 0.0 | 0.1 | 0.0 | 0.0  |
| PBMC PHA-L                     | 0.0 | 0.1 | 0.0 | 0.0  |
| Ramos (B cell) none            | 0.0 | 0.0 | 0.0 | 0.0  |
| Ramos (B cell) ionomycin       | 0.0 | 0.0 | 0.0 | 0.0  |
| B lymphocytes PWM              | 0.2 | 0.0 | 0.0 | 0.0  |
| B lymphocytes CD40L and IL-4   | 0.0 | 0.1 | 0.1 | 0.3  |
| EOL-1 dbcAMP                   | 0.2 | 0.2 | 0.4 | 0.0  |



|                                                    |      |      |      |      |
|----------------------------------------------------|------|------|------|------|
| EOL-1 dbcAMP<br>PMA/ionomycin                      | 0.1  | 0.4  | 0.2  | 0.6  |
| Dendritic cells none                               | 1.4  | 1.1  | 1.0  | 2.8  |
| Dendritic cells LPS                                | 0.3  | 0.4  | 0.3  | 0.4  |
| Dendritic cells anti-<br>CD40                      | 2.4  | 3.0  | 3.5  | 6.7  |
| Monocytes rest                                     | 0.8  | 0.8  | 0.6  | 1.3  |
| Monocytes LPS                                      | 0.0  | 0.0  | 0.3  | 0.0  |
| Macrophages rest                                   | 1.3  | 1.0  | 0.0  | 2.0  |
| Macrophages LPS                                    | 0.0  | 0.2  | 0.1  | 0.4  |
| HUVEC none                                         | 1.1  | 1.4  | 0.6  | 2.5  |
| HUVEC starved                                      | 4.4  | 4.7  | 2.9  | 6.0  |
| HUVEC IL-1beta                                     | 1.7  | 2.8  | 1.0  | 2.3  |
| HUVEC IFN gamma                                    | 1.6  | 1.4  | 2.5  | 1.9  |
| HUVEC TNF alpha +<br>IFN gamma                     | 0.3  | 0.3  | 0.5  | 0.5  |
| HUVEC TNF alpha +<br>IL4                           | 0.2  | 0.3  | 0.3  | 1.3  |
| HUVEC IL-11                                        | 0.9  | 1.2  | 2.2  | 0.5  |
| Lung Microvascular EC<br>none                      | 2.2  | 6.5  | 2.8  | 6.7  |
| Lung Microvascular EC<br>TNFalpha + IL-1beta       | 12.7 | 11.9 | 8.5  | 15.5 |
| Microvascular Dermal<br>EC none                    | 32.1 | 30.8 | 22.4 | 22.4 |
| Microvascular Dermal<br>EC TNFalpha + IL-<br>1beta | 16.3 | 16.2 | 8.8  | 14.4 |
| Bronchial epithelium<br>TNFalpha + IL1beta         | 24.0 | 31.2 | 15.1 | 50.7 |
| Small airway epithelium<br>none                    | 8.8  | 5.9  | 6.7  | 12.8 |
| Small airway epithelium<br>TNFalpha + IL-1beta     | 31.9 | 43.5 | 21.0 | 44.8 |
| Coronary artery SMC<br>rest                        | 27.4 | 28.7 | 8.5  | 35.8 |
| Coronary artery SMC<br>TNFalpha + IL-1beta         | 12.9 | 21.6 | 27.4 | 17.8 |
| Astrocytes rest                                    | 17.1 | 14.9 | 23.8 | 24.3 |
| Astrocytes TNFalpha +<br>IL-1beta                  | 32.8 | 29.5 | 28.1 | 35.1 |
| KU-812 (Basophil) rest                             | 1.0  | 1.8  | 1.3  | 0.7  |
| KU-812 (Basophil)<br>PMA/ionomycin                 | 1.4  | 3.3  | 2.0  | 3.7  |
| CCD1106                                            | 1.4  | 0.2  | 0.7  | 2.7  |

|                                                   |       |       |       |       |
|---------------------------------------------------|-------|-------|-------|-------|
| (Keratinocytes) none                              |       |       |       |       |
| CCD1106<br>(Keratinocytes)<br>TNFalpha + IL-1beta | 0.9   | 0.3   | 0.8   | 1.3   |
| Liver cirrhosis                                   | 2.9   | 3.0   | 2.4   | 4.8   |
| Lupus kidney                                      | 3.0   | 2.9   | 0.9   | 4.4   |
| NCI-H292 none                                     | 10.4  | 13.7  | 5.6   | 18.8  |
| NCI-H292 IL-4                                     | 14.2  | 14.9  | 6.8   | 17.1  |
| NCI-H292 IL-9                                     | 13.2  | 16.7  | 9.3   | 12.8  |
| NCI-H292 IL-13                                    | 9.4   | 8.6   | 15.9  | 9.0   |
| NCI-H292 IFN gamma                                | 3.8   | 4.7   | 4.7   | 5.3   |
| HPAEC none                                        | 1.2   | 1.0   | 1.6   | 2.8   |
| HPAEC TNF alpha +<br>IL-1 beta                    | 5.8   | 2.6   | 4.7   | 6.0   |
| Lung fibroblast none                              | 100.0 | 100.0 | 100.0 | 100.0 |
| Lung fibroblast TNF<br>alpha + IL-1 beta          | 8.5   | 12.2  | 15.9  | 15.2  |
| Lung fibroblast IL-4                              | 74.2  | 79.6  | 45.7  | 97.3  |
| Lung fibroblast IL-9                              | 27.7  | 48.6  | 30.6  | 50.3  |
| Lung fibroblast IL-13                             | 48.0  | 39.5  | 27.4  | 55.9  |
| Lung fibroblast IFN<br>gamma                      | 76.3  | 82.9  | 42.6  | 98.6  |
| Dermal fibroblast<br>CCD1070 rest                 | 52.9  | 56.3  | 27.2  | 65.5  |
| Dermal fibroblast<br>CCD1070 TNF alpha            | 33.9  | 42.6  | 19.8  | 46.7  |
| Dermal fibroblast<br>CCD1070 IL-1 beta            | 29.1  | 27.9  | 70.2  | 28.9  |
| Dermal fibroblast IFN<br>gamma                    | 6.1   | 3.6   | 8.9   | 7.9   |
| Dermal fibroblast IL-4                            | 14.5  | 16.2  | 17.3  | 18.9  |
| IBD Colitis 2                                     | 0.1   | 0.1   | 0.2   | 0.5   |
| IBD Crohn's                                       | 0.6   | 0.4   | 0.0   | 0.8   |
| Colon                                             | 7.6   | 6.4   | 8.0   | 11.3  |
| Lung                                              | 59.5  | 75.8  | 47.6  | 74.7  |
| Thymus                                            | 16.5  | 17.3  | 10.2  | 19.6  |
| Kidney                                            | 6.8   | 9.0   | 3.0   | 6.5   |

Table 24. Panel CNS\_1

| Tissue Name  | Rel. Exp.(%) Ag2263,<br>Run 171669090 | Tissue Name       | Rel. Exp.(%) Ag2263,<br>Run 171669090 |
|--------------|---------------------------------------|-------------------|---------------------------------------|
| BA4 Control  | 22.8                                  | BA17 PSP          | 11.2                                  |
| BA4 Control2 | 38.2                                  | BA17 PSP2         | 7.1                                   |
| BA4          | 3.7                                   | Sub Nigra Control | 100.0                                 |

|                   |      |                            |      |
|-------------------|------|----------------------------|------|
| Alzheimer's2      |      |                            |      |
| BA4 Parkinson's   | 45.7 | Sub Nigra Control2         | 51.8 |
| BA4 Parkinson's2  | 31.2 | Sub Nigra Alzheimer's2     | 30.8 |
| BA4 Huntington's  | 12.3 | Sub Nigra Parkinson's2     | 89.5 |
| BA4 Huntington's2 | 12.2 | Sub Nigra Huntington's     | 59.0 |
| BA4 PSP           | 13.6 | Sub Nigra Huntington's2    | 16.2 |
| BA4 PSP2          | 42.6 | Sub Nigra PSP2             | 22.5 |
| BA4 Depression    | 27.9 | Sub Nigra Depression       | 40.6 |
| BA4 Depression2   | 10.9 | Sub Nigra Depression2      | 12.8 |
| BA7 Control       | 28.3 | Glob Palladus Control      | 36.1 |
| BA7 Control2      | 27.2 | Glob Palladus Control2     | 21.3 |
| BA7 Alzheimer's2  | 5.5  | Glob Palladus Alzheimer's  | 26.1 |
| BA7 Parkinson's   | 13.2 | Glob Palladus Alzheimer's2 | 11.2 |
| BA7 Parkinson's2  | 12.8 | Glob Palladus Parkinson's  | 73.2 |
| BA7 Huntington's  | 14.8 | Glob Palladus Parkinson's2 | 15.7 |
| BA7 Huntington's2 | 22.2 | Glob Palladus PSP          | 15.0 |
| BA7 PSP           | 29.1 | Glob Palladus PSP2         | 10.4 |
| BA7 PSP2          | 8.9  | Glob Palladus Depression   | 28.3 |
| BA7 Depression    | 5.4  | Temp Pole Control          | 5.4  |
| BA9 Control       | 14.3 | Temp Pole Control2         | 25.2 |
| BA9 Control2      | 57.0 | Temp Pole Alzheimer's      | 10.0 |
| BA9 Alzheimer's   | 5.5  | Temp Pole Alzheimer's2     | 2.5  |
| BA9 Alzheimer's2  | 13.8 | Temp Pole Parkinson's      | 15.5 |
| BA9 Parkinson's   | 16.2 | Temp Pole Parkinson's2     | 27.9 |
| BA9 Parkinson's2  | 21.0 | Temp Pole Huntington's     | 22.4 |
| BA9               | 21.5 | Temp Pole PSP              | 1.3  |

|                       |      |                           |      |
|-----------------------|------|---------------------------|------|
| Huntington's          |      |                           |      |
| BA9<br>Huntington's2  | 11.9 | Temp Pole PSP2            | 6.4  |
| BA9 PSP               | 27.7 | Temp Pole<br>Depression2  | 12.3 |
| BA9 PSP2              | 5.9  | Cing Gyr Control          | 48.3 |
| BA9 Depression        | 11.0 | Cing Gyr Control2         | 28.1 |
| BA9<br>Depression2    | 9.5  | Cing Gyr<br>Alzheimer's   | 27.2 |
| BA17 Control          | 25.0 | Cing Gyr<br>Alzheimer's2  | 13.1 |
| BA17 Control2         | 45.7 | Cing Gyr Parkinson's      | 29.7 |
| BA17<br>Alzheimer's2  | 6.5  | Cing Gyr<br>Parkinson's2  | 37.4 |
| BA17<br>Parkinson's   | 35.4 | Cing Gyr<br>Huntington's  | 70.7 |
| BA17<br>Parkinson's2  | 15.3 | Cing Gyr<br>Huntington's2 | 32.1 |
| BA17<br>Huntington's  | 15.5 | Cing Gyr PSP              | 42.6 |
| BA17<br>Huntington's2 | 8.1  | Cing Gyr PSP2             | 8.3  |
| BA17<br>Depression    | 26.2 | Cing Gyr Depression       | 20.6 |
| BA17<br>Depression2   | 59.9 | Cing Gyr<br>Depression2   | 36.3 |

#### CNS\_neurodegeneration\_v1.0 Summary: Ag1848/Ag2263/Ag2422

Multiple experiments using different probe/primer sets produce results that are in good agreement. Highest expression of a NOV1 gene is detected in the occipital cortex of a control patient. Significant levels of expression are also detected in the hippocampus, inferior temporal cortex, and the superior temporal cortex of brain tissue from an Alzheimer's patient.

Based on its homology, a NOV1 gene product is most similar to an UNC5H receptor, which as a class is known to act both in axon guidance and neuronal migration during development, as well as in inducing apoptosis (except when stimulated by the ligand netrin-1).

Panel CNS\_Neurodegeneration\_V1.0 shows a moderate increase (1.5 to 2-fold) in the temporal cortex of the Alzheimer's disease brain when compared to non-demented elderly either with or without a high amyloid plaque load [this difference is apparent after scaling the RTQ-PCR data based upon overall RNA amount/quality, and is most apparent on Aq2263]. Thus NOV1 gene represents a protein that differentiates demented and non-demented elderly

who have a severe amyloid plaque load, making it an excellent drug target in Alzheimer's disease. The modulation and/or selective stimulation of this receptor may be of use in enhancing or directing compensatory synatogenesis and axon/dendritic outgrowth in response to neuronal death (stroke, head trauma) neurodegeneration (Alzheimer's, Parkinson's, Huntington's, spinocerebellar ataxia, progressive supranuclear palsy) or spinal cord injury. Furthermore, antagonism of this receptor may decrease apoptosis in Alzheimer's disease.

#### References:

1. Ellezam B, Selles-Navarro I, Manitt C, Kennedy TE, McKerracher L. Expression of netrin-1 and its receptors DCC and UNC-5H2 after axotomy and during regeneration of adult rat retinal ganglion cells. *Exp Neurol* 2001 Mar;168(1):105-15

Netrins are a family of chemotropic factors that guide axon outgrowth during development; however, their function in the adult CNS remains to be established. We examined the expression of the netrin receptors DCC and UNC5H2 in adult rat retinal ganglion cells (RGCs) after grafting a peripheral nerve (PN) to the transected optic nerve and following optic nerve transection alone. In situ hybridization revealed that both Dcc and Unc5h2 mRNAs are expressed by normal adult RGCs. In addition, netrin-1 was found to be constitutively expressed by RGCs. Quantitative analysis using in situ hybridization demonstrated that both Dcc and Unc5h2 were down-regulated by RGCs following axotomy. In the presence of an attached PN graft, Dcc and Unc5h2 were similarly down-regulated in surviving RGCs regardless of their success in regenerating an axon. Northern blot analysis demonstrated expression of netrin-1 in both optic and sciatic nerve, and Western blot analysis revealed the presence of netrin protein in both nerves. Immunohistochemical analysis indicated that netrin protein was closely associated with glial cells in the optic nerve. These results suggest that netrin-1, DCC, and UNC5H2 may contribute to regulating the regenerative capacity of adult RGCs.

2. Braisted JE, Catalano SM, Stimac R, Kennedy TE, Tessier-Lavigne M, Shatz CJ, O'Leary DD Netrin-1 promotes thalamic axon growth and is required for proper development of the thalamocortical projection. *J Neurosci* 2000 Aug 1;20(15):5792-801

The thalamocortical axon (TCA) projection originates in dorsal thalamus, conveys sensory input to the neocortex, and has a critical role in cortical development. We show that the secreted axon guidance molecule netrin-1 acts in vitro as an attractant and growth promoter for dorsal thalamic axons and is required for the proper development of the TCA projection in vivo. As TCAs approach the hypothalamus, they turn laterally into the ventral

telencephalon and extend toward the cortex through a population of netrin-1-expressing cells. DCC and neogenin, receptors implicated in mediating the attractant effects of netrin-1, are expressed in dorsal thalamus, whereas unc5h2 and unc5h3, netrin-1 receptors implicated in repulsion, are not. In vitro, dorsal thalamic axons show biased growth toward a source of netrin-1, which can be abolished by netrin-1-blocking antibodies. Netrin-1 also enhances overall axon outgrowth from explants of dorsal thalamus. The biased growth of dorsal thalamic axons toward the internal capsule zone of ventral telencephalic explants is attenuated, but not significantly, by netrin-1-blocking antibodies, suggesting that it releases another attractant activity for TCAs in addition to netrin-1. Analyses of netrin-1 <sup>-/-</sup> mice reveal that the TCA projection through the ventral telencephalon is disorganized, their pathway is abnormally restricted, and fewer dorsal thalamic axons reach cortex. These findings demonstrate that netrin-1 promotes the growth of TCAs through the ventral telencephalon and cooperates with other guidance cues to control their pathfinding from dorsal thalamus to cortex.

**Panel 1.2 Summary: Ag1522**

Expression of a NOV1 gene is highest in CNS cancer cell lines (CT=26.1). Of nine tissue samples derived from CNS cancer cell lines, expression of a NOV1 gene occurs in all samples, with expression high in three samples, moderate in five samples and low in one sample. High expression is also detectable in melanoma cell lines. Significant expression of a NOV1 gene is seen in gastric cancer and all ten samples of lung cancer cell lines in this sample. Thus, expression of a NOV1 gene could be used to distinguish those cancer cell lines from normal tissues. In addition, therapeutic modulation of the expression, or activity of a NOV1 gene product, might be of use in the treatment of melanoma, gastric cancer, lung cancer and brain cancer.

**Panel 1.3D Summary: Ag1522/Ag1848/Ag2263/Ag2422**

Four experiments using different probe/primer sets on the same tissue panel produce results that are in excellent agreement. In all four experiments, highest expression of a NOV1 gene is detected in CNS cancer cell lines. Expression is also significant in lung cancer and melanoma cell lines and in healthy brain tissue from the hippocampus and thalamus regions. Thus, the expression of a NOV1 gene could be used to distinguish these tissue samples from other samples. Moreover, therapeutic modulation of the expression, or function, of the CG50126-01 gene, through the use of small molecule drugs or antibodies, might be beneficial in the treatment of melanoma, lung cancer and brain cancer.

Among metabolic tissues, there is high expression of a NOV1 gene in adult heart tissue (CT=27.8) and moderate expression in fetal heart, adult and fetal liver, pancreas, adrenal gland, thyroid and pituitary. This widespread expression of a NOV1 gene product in tissues with metabolic function suggests a possible role for a NOV1 gene product in metabolic disorders, including obesity and diabetes.

The UNC5H receptors act both in axon guidance and neuronal migration during development, as well as inducers of apoptosis (except when stimulated by the ligand netrin-1). This panel shows widespread expression of a NOV1 gene in the central nervous system. Please see CNS\_neurodegeneration\_v1.0 for discussion of potential utility in the central nervous system.

**Panel 2D Summary: Ag1522/Ag1848/Ag2263/Ag2422**

Results from multiple experiments with four different probe and primer sets are in very good agreement. In all four experiments, highest expression of a NOV1 gene is detected in thyroid and ovarian cancers (CTs = 27-30), with lower expression also seen in most of the other tissues on this panel. Thus, the expression of a NOV1 gene could be used to distinguish ovarian and thyroid cancer cell lines from other tissues. Moreover, therapeutic modulation of the expression this gene, or its function, through the use of small molecule drugs or antibodies, might be of benefit in the treatment of ovarian and thyroid cancer. In addition, experiments with the probe and primer set Ag2263 show differential expression between samples derived from lung cancer and their adjacent normal tissues. Thus, expression of a NOV1 gene could be used to distinguish cancerous lung tissue from normal lung tissue. Moreover, therapeutic modulation of the expression or function of this gene or its protein product, through the use of antibodies or small molecule drugs, might be of benefit in the treatment of lung cancer.

**Panel 3D Summary: Ag2263**

Expression of a NOV1 gene occurs at moderate levels across all the tissues in this panel. Highest expression is detected in a small cell lung cancer (CT = 30.6) and neuroblastoma (CT = 30.7). In addition, significant expression is detected in a cluster of small cell lung cancer lines. Thus, this gene could be used to distinguish lung cancer cell lines from other samples. Moreover, therapeutic modulation of the CG50126-01 gene or its protein product, through the use of small molecule drugs or antibodies might be of benefit in the treatment of small cell lung cancer.

**Panel 4D Summary: Ag1522/Ag1848/Ag2263/Ag2422**

Experiments using each of the four probe and primer sets that correspond to a NOV1 gene produce results that are in excellent agreement. In all the experiments, expression of a NOV1 gene occurs at moderate to low levels in many of the tissues in the sample. Highest expression in each experiment occurs in lung fibroblasts (CT = 29). Moderate expression in lung fibroblasts treated with IL-4 is also consistent among all four experiments (CT = 30). Lower expression is also detected in a variety of fibroblasts, endothelial and smooth muscle cells. The expression of a NOV1 gene produces a complex profile; it is upregulated by TNF-alpha in small airway epithelium, but clearly downregulated by the same stimulus in lung fibroblasts. The gene most probably encodes a netrin receptor that may be important in understanding cell migration. Regulation of the protein encoded for by a NOV1 gene could potentially control the progression of keloid formation, emphysema and other conditions in which TNF-alpha and IL-1 beta are present and tissue remodeling may occur.

#### Panel CNS\_1 Summary: Ag2263

Expression of NOV1 is moderate to low across many of the tissues in this panel. Highest expression is detected in the substantia nigra (CT = 31.4). Although no disease-specific expression is seen in this panel, the expression profile confirms the expression of this gene in the central nervous system. Please see CNS\_neurodegeneration\_v1.0 for potential utility of the CG50126-01 gene regarding the CNS.

#### NOV2

Expression of gene CG50718-01 was assessed using the primer-probe sets Ag1555 and Ag2315, described in Tables 25 and 26. Results of the RTQ-PCR runs are shown in Tables 27, 28, 29 and 30.

**Table 25. Probe Name Ag1555**

| Primers | Sequences                                   | Length | Start Position | SEQ ID NO: |
|---------|---------------------------------------------|--------|----------------|------------|
| Forward | 5'-gaagtgaagaatgtgcatggt-3'                 | 22     | 6680           | 145        |
| Probe   | TET-5'-caccagtgcattctggatctcttatca-3'-TAMRA | 27     | 6730           | 146        |
| Reverse | 5'-tgggctgattacttccttatt-3'                 | 22     | 6757           | 147        |

**Table 26. Probe Name Ag2315**

| Primers | Sequences                                | Length | Start Position | SEQ ID NO: |
|---------|------------------------------------------|--------|----------------|------------|
| Forward | 5'-agatgagtcagtgccgtagc-3'               | 21     | 3711           | 148        |
| Probe   | TET-5'-cctccacaaaatttqactttaatcaactq-3'- | 29     | 3733           | 149        |



|         |                             |    |      |     |
|---------|-----------------------------|----|------|-----|
|         | TAMRA                       |    |      |     |
| Reverse | 5'-tccatttcagccatacaagtc-3' | 22 | 3769 | 150 |

Table 27. Panel 1.3D

| Tissue Name                    | Rel.<br>Exp.(%)<br>Ag1555,<br>Run<br>146380268 | Rel.<br>Exp.(%)<br>Ag1555,<br>Run<br>147775028 | Rel.<br>Exp.(%)<br>Ag2315,<br>Run<br>159198312 | Tissue Name                             | Rel.<br>Exp.(%)<br>Ag1555,<br>Run<br>146380268 | Rel.<br>Exp.(%)<br>Ag1555,<br>Run<br>147775028 | Rel.<br>Exp.(%)<br>Ag2315,<br>Run<br>159198312 |
|--------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|-----------------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|
| Liver<br>adenocarcinoma        | 0.0                                            | 0.0                                            | 0.0                                            | Kidney<br>(fetal)                       | 33.9                                           | 37.6                                           | 90.8                                           |
| Pancreas                       | 5.8                                            | 1.6                                            | 3.9                                            | Renal ca.<br>786-0                      | 0.0                                            | 0.0                                            | 0.0                                            |
| Pancreatic ca.<br>CAPAN 2      | 0.0                                            | 0.0                                            | 0.0                                            | Renal ca.<br>A498                       | 0.0                                            | 0.0                                            | 0.0                                            |
| Adrenal gland                  | 0.0                                            | 1.9                                            | 0.0                                            | Renal ca.<br>RXF 393                    | 0.0                                            | 0.0                                            | 0.0                                            |
| Thyroid                        | 8.3                                            | 24.7                                           | 25.3                                           | Renal ca.<br>ACHN                       | 0.0                                            | 0.0                                            | 0.0                                            |
| Salivary gland                 | 1.0                                            | 0.0                                            | 0.0                                            | Renal ca.<br>UO-31                      | 0.0                                            | 1.4                                            | 0.0                                            |
| Pituitary gland                | 0.0                                            | 0.0                                            | 8.0                                            | Renal ca.<br>TK-10                      | 0.0                                            | 0.0                                            | 0.0                                            |
| Brain (fetal)                  | 0.6                                            | 0.0                                            | 3.8                                            | Liver                                   | 0.0                                            | 0.0                                            | 0.0                                            |
| Brain (whole)                  | 1.3                                            | 1.6                                            | 3.3                                            | Liver (fetal)                           | 0.0                                            | 3.6                                            | 0.0                                            |
| Brain<br>(amygdala)            | 3.4                                            | 4.0                                            | 6.7                                            | Liver ca.<br>(hepatoblast)<br>HepG2     | 0.0                                            | 0.0                                            | 0.0                                            |
| Brain<br>(cerebellum)          | 0.0                                            | 0.0                                            | 0.0                                            | Lung                                    | 51.1                                           | 52.5                                           | 70.7                                           |
| Brain<br>(hippocampus)         | 1.2                                            | 0.6                                            | 5.9                                            | Lung (fetal)                            | 100.0                                          | 100.0                                          | 74.2                                           |
| Brain<br>(substantia<br>nigra) | 0.0                                            | 0.0                                            | 0.0                                            | Lung ca.<br>(small cell)<br>LX-1        | 0.0                                            | 0.0                                            | 0.0                                            |
| Brain<br>(thalamus)            | 3.2                                            | 1.3                                            | 4.0                                            | Lung ca.<br>(small cell)<br>NCI-H69     | 2.4                                            | 0.0                                            | 32.5                                           |
| Cerebral Cortex                | 0.0                                            | 0.0                                            | 12.4                                           | Lung ca.<br>(s.cell var.)<br>SHP-77     | 0.0                                            | 0.0                                            | 10.7                                           |
| Spinal cord                    | 1.1                                            | 0.0                                            | 0.0                                            | Lung ca.<br>(large<br>cell)NCI-<br>H460 | 0.0                                            | 0.0                                            | 0.0                                            |
| glio/astro U87-<br>MG          | 0.0                                            | 2.7                                            | 0.0                                            | Lung ca.<br>(non-sm.<br>cell) A549      | 0.0                                            | 0.0                                            | 0.0                                            |
| glio/astro U-<br>118-MG        | 27.2                                           | 34.6                                           | 15.8                                           | Lung ca.<br>(non-s.cell)<br>NCI-H23     | 0.0                                            | 0.0                                            | 0.0                                            |

|                                    |     |      |      |                                          |      |      |      |
|------------------------------------|-----|------|------|------------------------------------------|------|------|------|
| astrocytoma<br>SW1783              | 5.4 | 13.8 | 16.0 | Lung ca.<br>(non-s.cell)<br>HOP-62       | 0.7  | 0.9  | 0.0  |
| neuro*; met<br>SK-N-AS             | 0.0 | 0.6  | 0.0  | Lung ca.<br>(non-s.cl)<br>NCI-H522       | 9.9  | 5.4  | 20.9 |
| astrocytoma SF-<br>539             | 0.8 | 0.0  | 0.0  | Lung ca.<br>(squam.) SW<br>900           | 0.0  | 0.0  | 0.0  |
| astrocytoma<br>SNB-75              | 0.0 | 0.0  | 0.0  | Lung ca.<br>(squam.)<br>NCI-H596         | 1.3  | 2.2  | 9.0  |
| glioma SNB-19                      | 0.0 | 0.0  | 0.0  | Mammary<br>gland                         | 13.0 | 26.6 | 11.5 |
| glioma U251                        | 0.0 | 0.0  | 0.0  | Breast ca.*<br>(pl.ef) MCF-<br>7         | 3.9  | 0.9  | 15.6 |
| glioma SF-295                      | 1.3 | 3.3  | 0.0  | Breast ca.*<br>(pl.ef)<br>MDA-MB-<br>231 | 0.0  | 0.0  | 0.0  |
| Heart (Fetal)                      | 0.0 | 0.0  | 7.4  | Breast ca.*<br>(pl. ef)<br>T47D          | 0.0  | 0.0  | 6.1  |
| Heart                              | 0.0 | 5.7  | 0.0  | Breast ca.<br>BT-549                     | 0.0  | 0.0  | 0.0  |
| Skeletal muscle<br>(Fetal)         | 3.5 | 1.6  | 15.1 | Breast ca.<br>MDA-N                      | 0.0  | 0.0  | 0.0  |
| Skeletal muscle                    | 0.0 | 1.4  | 2.5  | Ovary                                    | 5.2  | 1.6  | 5.8  |
| Bone marrow                        | 1.0 | 4.1  | 0.0  | Ovarian ca.<br>OVCAR-3                   | 0.0  | 0.0  | 6.7  |
| Thymus                             | 1.0 | 0.0  | 8.8  | Ovarian ca.<br>OVCAR-4                   | 0.0  | 0.0  | 0.0  |
| Spleen                             | 0.0 | 0.0  | 0.0  | Ovarian ca.<br>OVCAR-5                   | 0.0  | 0.0  | 0.0  |
| Lymph node                         | 3.7 | 4.8  | 7.1  | Ovarian ca.<br>OVCAR-8                   | 0.0  | 0.0  | 0.0  |
| Colorectal                         | 0.0 | 0.0  | 0.0  | Ovarian ca.<br>IGROV-1                   | 0.0  | 0.0  | 0.0  |
| Stomach                            | 1.2 | 2.3  | 0.0  | Ovarian ca.<br>(ascites) SK-<br>OV-3     | 0.0  | 0.0  | 0.0  |
| Small intestine                    | 2.2 | 6.7  | 0.0  | Uterus                                   | 0.0  | 0.9  | 0.0  |
| Colon ca.<br>SW480                 | 0.0 | 0.0  | 0.0  | Placenta                                 | 11.8 | 27.7 | 23.7 |
| Colon ca.*<br>SW620 (SW480<br>met) | 0.0 | 0.0  | 0.0  | Prostate                                 | 3.5  | 0.9  | 3.0  |
| Colon ca. HT29                     | 0.0 | 0.0  | 0.0  | Prostate ca.*<br>(bone met)<br>PC-3      | 0.0  | 0.0  | 0.0  |

|                                 |      |      |      |                            |      |      |       |
|---------------------------------|------|------|------|----------------------------|------|------|-------|
| Colon ca. HCT-116               | 0.0  | 0.0  | 2.7  | Testis                     | 58.2 | 67.4 | 21.5  |
| Colon ca. CaCo-2                | 0.0  | 0.0  | 0.0  | Melanoma Hs688(A).T        | 22.7 | 52.1 | 18.2  |
| CC Well to Mod Diff (ODO3866)   | 0.0  | 0.0  | 0.0  | Melanoma* (met) Hs688(B).T | 4.8  | 4.2  | 0.0   |
| Colon ca. HCC-2998              | 0.0  | 0.0  | 0.0  | Melanoma UACC-62           | 0.0  | 1.5  | 0.0   |
| Gastric ca. (liver met) NCI-N87 | 0.0  | 0.0  | 0.0  | Melanoma M14               | 0.0  | 0.0  | 0.0   |
| Bladder                         | 2.0  | 0.0  | 6.1  | Melanoma LOX IMVI          | 0.0  | 0.0  | 0.0   |
| Trachea                         | 2.4  | 3.6  | 0.0  | Melanoma* (met) SK-MEL-5   | 0.0  | 0.0  | 0.0   |
| Kidney                          | 15.5 | 17.8 | 22.2 | Adipose                    | 38.2 | 40.6 | 100.0 |

Table 28. Panel 2D

| Tissue Name                    | Rel. Exp.(%)<br>Ag1555,<br>Run<br>147775063 | Rel. Exp.(%)<br>Ag1555,<br>Run<br>159601974 | Rel. Exp.(%)<br>Ag2315,<br>Run<br>159200827 | Tissue Name           | Rel. Exp.(%)<br>Ag1555,<br>Run<br>147775063 | Rel. Exp.(%)<br>Ag1555,<br>Run<br>159601974 | Rel. Exp.(%)<br>Ag2315,<br>Run<br>159200827 |
|--------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|-----------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| Normal Colon                   | 3.8                                         | 7.1                                         | 12.4                                        | Kidney Margin 8120608 | 2.9                                         | 1.2                                         | 1.7                                         |
| CC Well to Mod Diff (ODO3866)  | 1.0                                         | 0.0                                         | 2.3                                         | Kidney Cancer 8120613 | 0.0                                         | 0.0                                         | 0.0                                         |
| CC Margin (ODO3866)            | 0.0                                         | 0.0                                         | 0.7                                         | Kidney Margin 8120614 | 1.2                                         | 2.6                                         | 1.8                                         |
| CC Gr.2 rectosigmoid (ODO3868) | 0.0                                         | 0.0                                         | 0.0                                         | Kidney Cancer 9010320 | 2.7                                         | 2.6                                         | 2.1                                         |
| CC Margin (ODO3868)            | 0.0                                         | 0.7                                         | 0.0                                         | Kidney Margin 9010321 | 6.6                                         | 5.9                                         | 4.9                                         |
| CC Mod Diff (ODO3920)          | 0.0                                         | 0.0                                         | 0.0                                         | Normal Uterus         | 0.0                                         | 0.0                                         | 1.8                                         |
| CC Margin (ODO3920)            | 0.0                                         | 0.0                                         | 2.2                                         | Uterine Cancer 064011 | 0.0                                         | 0.0                                         | 4.5                                         |
| CC Gr.2 ascend colon (ODO3921) | 0.0                                         | 0.0                                         | 0.0                                         | Normal Thyroid        | 34.9                                        | 27.4                                        | 11.4                                        |
| CC Margin (ODO3921)            | 0.0                                         | 0.0                                         | 0.0                                         | Thyroid Cancer        | 2.9                                         | 7.2                                         | 7.9                                         |
| CC from                        | 1.6                                         | 1.1                                         | 0.0                                         | Thyroid               | 1.3                                         | 3.3                                         | 2.0                                         |

|                                             |      |      |      |                                              |      |      |      |
|---------------------------------------------|------|------|------|----------------------------------------------|------|------|------|
| Partial<br>Hepatectomy<br>(ODO4309)<br>Mets |      |      |      | Cancer<br>A302152                            |      |      |      |
| Liver Margin<br>(ODO4309)                   | 0.0  | 0.0  | 2.0  | Thyroid<br>Margin<br>A302153                 | 49.7 | 69.7 | 72.2 |
| Colon mets to<br>lung<br>(OD04451-<br>01)   | 2.0  | 1.0  | 0.5  | Normal<br>Breast                             | 10.0 | 8.9  | 25.3 |
| Lung Margin<br>(OD04451-<br>02)             | 8.6  | 10.0 | 10.9 | Breast<br>Cancer                             | 10.2 | 3.0  | 1.1  |
| Normal<br>Prostate<br>6546-1                | 4.2  | 12.2 | 1.4  | Breast<br>Cancer<br>(OD04590-<br>01)         | 0.0  | 2.8  | 3.9  |
| Prostate<br>Cancer<br>(OD04410)             | 0.0  | 0.0  | 3.4  | Breast<br>Cancer<br>Mets<br>(OD04590-<br>03) | 7.8  | 7.3  | 7.9  |
| Prostate<br>Margin<br>(OD04410)             | 0.8  | 6.4  | 2.2  | Breast<br>Cancer<br>Metastasis               | 4.1  | 8.0  | 3.5  |
| Prostate<br>Cancer<br>(OD04720-<br>01)      | 9.5  | 11.7 | 19.6 | Breast<br>Cancer                             | 0.0  | 0.0  | 1.2  |
| Prostate<br>Margin<br>(OD04720-<br>02)      | 10.0 | 11.3 | 24.5 | Breast<br>Cancer                             | 3.7  | 2.9  | 0.9  |
| Normal Lung                                 | 59.9 | 61.1 | 87.7 | Breast<br>Cancer<br>9100266                  | 2.2  | 1.1  | 1.5  |
| Lung Met to<br>Muscle<br>(ODO4286)          | 0.0  | 0.0  | 0.0  | Breast<br>Margin<br>9100265                  | 0.0  | 0.0  | 0.5  |
| Muscle<br>Margin<br>(ODO4286)               | 0.9  | 0.0  | 1.8  | Breast<br>Cancer<br>A209073                  | 0.7  | 1.1  | 1.9  |
| Lung<br>Malignant<br>Cancer<br>(OD03126)    | 1.9  | 2.8  | 1.7  | Breast<br>Margin<br>A2090734                 | 0.0  | 1.2  | 0.9  |
| Lung Margin<br>(OD03126)                    | 36.3 | 35.6 | 43.8 | Normal<br>Liver                              | 0.0  | 0.0  | 0.0  |
| Lung Cancer<br>(OD04404)                    | 2.2  | 4.4  | 4.3  | Liver<br>Cancer                              | 0.0  | 0.0  | 0.6  |
| Lung Margin                                 | 9.5  | 4.2  | 8.4  | Liver                                        | 0.0  | 0.0  | 0.0  |

|                                                |       |       |       |                                                   |     |     |     |
|------------------------------------------------|-------|-------|-------|---------------------------------------------------|-----|-----|-----|
| (OD04404)                                      |       |       |       | Cancer<br>1025                                    |     |     |     |
| Lung Cancer<br>(OD04565)                       | 0.0   | 0.0   | 0.0   | Liver<br>Cancer<br>1026                           | 0.0 | 0.0 | 0.0 |
| Lung Margin<br>(OD04565)                       | 10.8  | 9.7   | 14.1  | Liver<br>Cancer<br>6004-T                         | 0.0 | 1.0 | 0.6 |
| Lung Cancer<br>(OD04237-<br>01)                | 0.0   | 0.0   | 0.0   | Liver<br>Tissue<br>6004-N                         | 0.0 | 0.0 | 0.0 |
| Lung Margin<br>(OD04237-<br>02)                | 30.1  | 18.4  | 29.3  | Liver<br>Cancer<br>6005-T                         | 0.0 | 0.0 | 0.0 |
| Ocular Mel<br>Met to Liver<br>(ODO4310)        | 0.0   | 0.0   | 0.6   | Liver<br>Tissue<br>6005-N                         | 0.0 | 0.0 | 0.0 |
| Liver Margin<br>(ODO4310)                      | 1.0   | 2.0   | 0.0   | Normal<br>Bladder                                 | 4.7 | 2.2 | 2.9 |
| Melanoma<br>Metastasis                         | 0.0   | 0.0   | 0.0   | Bladder<br>Cancer                                 | 0.0 | 0.0 | 0.0 |
| Lung Margin<br>(OD04321)                       | 25.7  | 47.0  | 49.0  | Bladder<br>Cancer                                 | 0.0 | 4.2 | 5.5 |
| Normal<br>Kidney                               | 86.5  | 100.0 | 100.0 | Bladder<br>Cancer<br>(OD04718-<br>01)             | 0.7 | 1.6 | 1.1 |
| Kidney Ca,<br>Nuclear grade<br>2 (OD04338)     | 2.2   | 0.0   | 1.1   | Bladder<br>Normal<br>Adjacent<br>(OD04718-<br>03) | 4.4 | 0.9 | 6.3 |
| Kidney<br>Margin<br>(OD04338)                  | 55.1  | 35.8  | 58.2  | Normal<br>Ovary                                   | 1.7 | 0.0 | 0.9 |
| Kidney Ca<br>Nuclear grade<br>1/2<br>(OD04339) | 0.0   | 0.0   | 0.0   | Ovarian<br>Cancer                                 | 0.0 | 4.2 | 3.3 |
| Kidney<br>Margin<br>(OD04339)                  | 77.9  | 63.7  | 77.9  | Ovarian<br>Cancer<br>(OD04768-<br>07)             | 0.0 | 0.0 | 0.0 |
| Kidney Ca,<br>Clear cell<br>type<br>(OD04340)  | 1.7   | 0.0   | 0.0   | Ovary<br>Margin<br>(OD04768-<br>08)               | 9.4 | 5.5 | 6.9 |
| Kidney<br>Margin<br>(OD04340)                  | 100.0 | 53.2  | 62.4  | Normal<br>Stomach                                 | 0.0 | 0.0 | 0.0 |
| Kidney Ca,<br>Nuclear grade                    | 25.9  | 23.2  | 0.0   | Gastric<br>Cancer                                 | 0.0 | 0.0 | 1.5 |

|                            |      |      |      |                        |     |     |     |
|----------------------------|------|------|------|------------------------|-----|-----|-----|
| 3 (OD04348)                |      |      |      | 9060358                |     |     |     |
| Kidney Margin (OD04348)    | 40.9 | 50.3 | 54.7 | Stomach Margin 9060359 | 0.0 | 0.0 | 2.0 |
| Kidney Cancer (OD04622-01) | 0.6  | 0.0  | 0.0  | Gastric Cancer 9060395 | 0.9 | 1.2 | 1.8 |
| Kidney Margin (OD04622-03) | 0.0  | 0.0  | 1.4  | Stomach Margin 9060394 | 0.0 | 1.0 | 0.7 |
| Kidney Cancer (OD04450-01) | 0.0  | 0.0  | 0.0  | Gastric Cancer 9060397 | 0.0 | 0.0 | 0.0 |
| Kidney Margin (OD04450-03) | 40.3 | 51.1 | 50.7 | Stomach Margin 9060396 | 0.0 | 0.0 | 0.0 |
| Kidney Cancer 8120607      | 0.0  | 0.0  | 0.0  | Gastric Cancer 064005  | 0.0 | 0.0 | 2.5 |

Table 29. Panel 4D

| Tissue Name        | Rel. Exp.(%)<br>Ag1555, Run<br>147775116 | Rel. Exp.(%)<br>Ag2315, Run<br>159202089 | Tissue Name                                 | Rel. Exp.(%)<br>Ag1555, Run<br>147775116 | Rel. Exp.(%)<br>Ag2315, Run<br>159202089 |
|--------------------|------------------------------------------|------------------------------------------|---------------------------------------------|------------------------------------------|------------------------------------------|
| Secondary Th1 act  | 0.0                                      | 0.0                                      | HUVEC IL-1beta                              | 0.0                                      | 0.0                                      |
| Secondary Th2 act  | 0.0                                      | 0.0                                      | HUVEC IFN gamma                             | 0.0                                      | 0.0                                      |
| Secondary Tr1 act  | 0.0                                      | 0.7                                      | HUVEC TNF alpha + IFN gamma                 | 0.0                                      | 0.0                                      |
| Secondary Th1 rest | 0.0                                      | 0.0                                      | HUVEC TNF alpha + IL4                       | 0.0                                      | 0.0                                      |
| Secondary Th2 rest | 0.0                                      | 0.0                                      | HUVEC IL-11                                 | 0.0                                      | 0.0                                      |
| Secondary Tr1 rest | 0.0                                      | 0.0                                      | Lung Microvascular EC none                  | 0.0                                      | 0.0                                      |
| Primary Th1 act    | 0.0                                      | 0.0                                      | Lung Microvascular EC TNFalpha + IL-1beta   | 0.0                                      | 0.0                                      |
| Primary Th2 act    | 0.0                                      | 0.0                                      | Microvascular Dermal EC none                | 0.0                                      | 0.0                                      |
| Primary Tr1 act    | 0.0                                      | 0.0                                      | Microvascular Dermal EC TNFalpha + IL-1beta | 0.0                                      | 0.0                                      |
| Primary Th1 rest   | 0.0                                      | 0.0                                      | Bronchial epithelium TNFalpha + IL1beta     | 0.0                                      | 0.0                                      |

|                                |     |     |                                             |     |     |
|--------------------------------|-----|-----|---------------------------------------------|-----|-----|
| Primary Th2 rest               | 0.0 | 0.0 | Small airway epithelium none                | 0.0 | 0.0 |
| Primary Tr1 rest               | 0.0 | 0.0 | Small airway epithelium TNFalpha + IL-1beta | 0.0 | 0.0 |
| CD45RA CD4 lymphocyte act      | 3.3 | 5.0 | Coronary artery SMC rest                    | 1.0 | 2.3 |
| CD45RO CD4 lymphocyte act      | 0.0 | 0.0 | Coronary artery SMC TNFalpha + IL-1beta     | 3.7 | 1.2 |
| CD8 lymphocyte act             | 0.0 | 0.0 | Astrocytes rest                             | 3.2 | 0.5 |
| Secondary CD8 lymphocyte rest  | 0.0 | 0.0 | Astrocytes TNFalpha + IL-1beta              | 1.0 | 1.5 |
| Secondary CD8 lymphocyte act   | 0.0 | 0.0 | KU-812 (Basophil) rest                      | 0.0 | 0.0 |
| CD4 lymphocyte none            | 0.0 | 0.0 | KU-812 (Basophil) PMA/ionomycin             | 0.0 | 0.0 |
| 2ry Th1/Th2/Tr1_anti-CD95 CH11 | 0.0 | 0.0 | CCD1106 (Keratinocytes) none                | 0.0 | 0.0 |
| LAK cells rest                 | 0.0 | 0.0 | CCD1106 (Keratinocytes) TNFalpha + IL-1beta | 0.0 | 0.0 |
| LAK cells IL-2                 | 0.0 | 0.0 | Liver cirrhosis                             | 1.4 | 3.8 |
| LAK cells IL-2+IL-12           | 0.0 | 0.0 | Lupus kidney                                | 0.0 | 0.8 |
| LAK cells IL-2+IFN gamma       | 0.0 | 0.0 | NCI-H292 none                               | 0.0 | 0.0 |
| LAK cells IL-2+ IL-18          | 0.0 | 0.0 | NCI-H292 IL-4                               | 0.0 | 2.3 |
| LAK cells PMA/ionomycin        | 0.0 | 0.0 | NCI-H292 IL-9                               | 0.0 | 0.5 |
| NK Cells IL-2 rest             | 0.0 | 0.0 | NCI-H292 IL-13                              | 0.0 | 1.3 |
| Two Way MLR 3 day              | 0.0 | 0.0 | NCI-H292 IFN gamma                          | 0.0 | 0.0 |
| Two Way MLR 5 day              | 0.0 | 0.0 | HPAEC none                                  | 0.0 | 0.0 |
| Two Way MLR 7 day              | 0.0 | 0.0 | HPAEC TNF alpha + IL-1 beta                 | 0.0 | 0.0 |
| PBMC rest                      | 0.0 | 0.0 | Lung fibroblast none                        | 0.0 | 0.9 |
| PBMC PWM                       | 0.0 | 0.0 | Lung fibroblast TNF alpha + IL-1 beta       | 0.0 | 0.0 |
| PBMC PHA-L                     | 0.0 | 0.0 | Lung fibroblast IL-4                        | 0.0 | 0.0 |
| Ramos (B cell) none            | 0.0 | 0.0 | Lung fibroblast IL-9                        | 5.7 | 1.3 |
| Ramos (B cell)                 | 0.0 | 0.0 | Lung fibroblast IL-                         | 1.5 | 1.5 |

|                              |     |     |                                     |       |       |
|------------------------------|-----|-----|-------------------------------------|-------|-------|
| ionomycin                    |     |     | 13                                  |       |       |
| B lymphocytes PWM            | 0.0 | 0.0 | Lung fibroblast IFN gamma           | 0.0   | 1.7   |
| B lymphocytes CD40L and IL-4 | 0.0 | 0.0 | Dermal fibroblast CCD1070 rest      | 12.9  | 17.2  |
| EOL-1 dbcAMP                 | 0.0 | 0.0 | Dermal fibroblast CCD1070 TNF alpha | 18.6  | 12.0  |
| EOL-1 dbcAMP PMA/ionomycin   | 0.0 | 0.0 | Dermal fibroblast CCD1070 IL-1 beta | 6.1   | 2.9   |
| Dendritic cells none         | 0.0 | 0.0 | Dermal fibroblast IFN gamma         | 0.0   | 0.0   |
| Dendritic cells LPS          | 0.0 | 0.0 | Dermal fibroblast IL-4              | 1.4   | 0.6   |
| Dendritic cells anti-CD40    | 0.0 | 0.0 | IBD Colitis 2                       | 0.0   | 1.4   |
| Monocytes rest               | 0.0 | 0.0 | IBD Crohn's                         | 0.0   | 0.0   |
| Monocytes LPS                | 0.0 | 0.0 | Colon                               | 0.6   | 0.0   |
| Macrophages rest             | 0.0 | 0.0 | Lung                                | 4.0   | 11.7  |
| Macrophages LPS              | 0.0 | 0.0 | Thymus                              | 100.0 | 100.0 |
| HUVEC none                   | 0.0 | 0.0 | Kidney                              | 4.2   | 5.3   |
| HUVEC starved                | 0.0 | 0.0 |                                     |       |       |

Table 30. Panel 5D

| Tissue Name                        | Rel. Exp.(%)<br>Ag2315, Run<br>169275446 | Tissue Name                                | Rel. Exp.(%)<br>Ag2315, Run<br>169275446 |
|------------------------------------|------------------------------------------|--------------------------------------------|------------------------------------------|
| 97457_Patient-02go_adipose         | 84.1                                     | 94709_Donor 2 AM - A_adipose               | 13.6                                     |
| 97476_Patient-07sk_skeletal muscle | 0.6                                      | 94710_Donor 2 AM - B_adipose               | 9.3                                      |
| 97477_Patient-07ut_uterus          | 0.0                                      | 94711_Donor 2 AM - C_adipose               | 3.6                                      |
| 97478_Patient-07pl_placenta        | 7.2                                      | 94712_Donor 2 AD - A_adipose               | 8.7                                      |
| 97481_Patient-08sk_skeletal muscle | 4.4                                      | 94713_Donor 2 AD - B_adipose               | 17.1                                     |
| 97482_Patient-08ut_uterus          | 0.5                                      | 94714_Donor 2 AD - C_adipose               | 21.6                                     |
| 97483_Patient-08pl_placenta        | 6.5                                      | 94742_Donor 3 U - A_Mesenchymal Stem Cells | 9.0                                      |
| 97486_Patient-09sk_skeletal muscle | 0.0                                      | 94743_Donor 3 U - B_Mesenchymal Stem Cells | 7.3                                      |
| 97487_Patient-09ut_uterus          | 0.5                                      | 94730_Donor 3 AM - A_adipose               | 14.8                                     |
| 97488_Patient-09pl_placenta        | 6.1                                      | 94731_Donor 3 AM - B_adipose               | 13.9                                     |
| 97492_Patient-10ut_uterus          | 0.0                                      | 94732_Donor 3 AM - C_adipose               | 5.9                                      |



|                                            |       |                                             |     |
|--------------------------------------------|-------|---------------------------------------------|-----|
| 97493_Patient-10pl_placenta                | 7.8   | 94733_Donor 3 AD - A_adipose                | 5.4 |
| 97495_Patient-11go_adipose                 | 100.0 | 94734_Donor 3 AD - B_adipose                | 4.7 |
| 97496_Patient-11sk_skeletal muscle         | 0.6   | 94735_Donor 3 AD - C_adipose                | 9.3 |
| 97497_Patient-11ut_uterus                  | 1.0   | 77138_Liver_HepG2untreated                  | 6.9 |
| 97498_Patient-11pl_placenta                | 7.3   | 73556_Heart_Cardiac stromal cells (primary) | 0.0 |
| 97500_Patient-12go_adipose                 | 61.6  | 81735_Small Intestine                       | 1.5 |
| 97501_Patient-12sk_skeletal muscle         | 3.2   | 72409_Kidney_Proximal Convoluted Tubule     | 0.0 |
| 97502_Patient-12ut_uterus                  | 1.4   | 82685_Small intestine_Duodenum              | 0.0 |
| 97503_Patient-12pl_placenta                | 1.5   | 90650_Adrenal_Adrenocortical adenoma        | 0.0 |
| 94721_Donor 2 U - A_Mesenchymal Stem Cells | 14.4  | 72410_Kidney_HRCE                           | 0.0 |
| 94722_Donor 2 U - B_Mesenchymal Stem Cells | 6.7   | 72411_Kidney_HRE                            | 0.0 |
| 94723_Donor 2 U - C_Mesenchymal Stem Cells | 6.0   | 73139_Uterus_Uterine smooth muscle cells    | 0.0 |

**Panel 1.3D Summary:** Ag1555/2315 Highest expression of the CG50718-01 gene is seen in adipose and the fetal lung (CTs=31.8-34.4). Results from three experiments with two different probe and primer sets produce similar expression profiles. Low but significant expression is also seen in the thyroid. Biologic cross-talk between the thyroid and adipose tissue is believed to be a component of some forms of obesity. Thus, the CG50718-01 gene product may be an important small molecule target for the treatment of obesity or other metabolic disorders.

In addition, the CG50718-01 gene appears to be expressed at significant levels in lung and kidney tissues from both fetal and adult sources, but not in any samples derived from lung or kidney cancer cell lines. Thus, expression of this gene could potentially be used to differentiate between normal lung and kidney tissue and lung and kidney cancer. Furthermore, therapeutic modulation of the CG50718-01 gene product may be beneficial in the treatment of lung and kidney cancers.

Please note that two other experiments with the probe and primer set Ag2315 had low/undetectable levels of expression in all the samples on this panel. (Data not shown.)

**Panel 2D Summary:** Ag1555/2315 Three experiments with two different probe and primer sets produce results that are in excellent agreement with highest expression of the CG50718-01 gene in normal kidney tissue (CTs=30.7-32.4). There are also significant levels of expression in samples derived from normal lung tissue, a result that is in concordance with the expression seen in Panel 1.3D. This gene appears to be preferentially expressed in healthy tissue, when compared to adjacent cancerous tissue. Thus, expression of the CG50718-01 gene could be used to distinguish normal kidney and lung tissue from malignant kidney and lung tissue. Moreover, therapeutic modulation of this gene, through small molecule drugs, antibodies or protein therapeutics might be of benefit in the treatment of kidney cancer and lung cancer.

**Panel 3D Summary:** Ag2315 Expression is low/undetectable in all the samples in this panel (CT>35). (Data not shown.)

**Panel 4D Summary:** Ag1555/Ag2315 The CG50718-01 transcript is detected at significant levels in the thymus (CT 31.48) and at lower levels in dermal fibroblasts (CT 33.91). This transcript encodes a protein that could potentially serve as a marker for thymus tissue and may also be involved in skin homeostasis. Therapeutics designed with the protein encoded by the CG50718-01 transcript could be important for maintaining or restoring normal function to these organs during inflammation.

**Panel 5D Summary:** Ag2315 is modestly expressed (CT values 31-34) in human adipose tissue and in cultured human adipocytes. This expression is in agreement with the significant levels of expression in adipose detected in Panel 1.3D. Thus, this gene product may be a small molecule target for the treatment of obesity.

### NOV3

Expression of NOV3 was assessed using the primer-probe set Ag2304, described in Table 31. Results of the RTQ-PCR runs are shown in Tables 32, 33, 34 and 35.

**Table 31. Probe Name Ag2304**

| Primers | Sequences                                          | Length | Start Position | SEQ ID NO: |
|---------|----------------------------------------------------|--------|----------------|------------|
| Forward | 5'-accttaagtcctgccaacaatt-3'                       | 22     | 4100           | 151        |
| Probe   | TET-5'-<br>ttacagagtccaaattgtggatccca-3'-<br>TAMRA | 26     | 4147           | 152        |
| Reverse | 5'-tgatcccttccagaatttgac-3'                        | 21     | 4173           | 153        |

**Table 32. CNS\_neurodegeneration\_v1.0**

| Tissue Name | Rel. Exp.(%) Ag2304. Run | Tissue Name | Rel. Exp.(%) Ag2304. Run |
|-------------|--------------------------|-------------|--------------------------|
|-------------|--------------------------|-------------|--------------------------|

|                                  | 206262286 |                                   | 206262286 |
|----------------------------------|-----------|-----------------------------------|-----------|
| AD 1 Hippo                       | 0.0       | Control (Path) 3<br>Temporal Ctx  | 7.3       |
| AD 2 Hippo                       | 38.4      | Control (Path) 4<br>Temporal Ctx  | 29.5      |
| AD 3 Hippo                       | 8.5       | AD 1 Occipital Ctx                | 16.5      |
| AD 4 Hippo                       | 9.5       | AD 2 Occipital Ctx<br>(Missing)   | 0.0       |
| AD 5 Hippo                       | 100.0     | AD 3 Occipital Ctx                | 8.7       |
| AD 6 Hippo                       | 70.7      | AD 4 Occipital Ctx                | 22.2      |
| Control 2 Hippo                  | 44.8      | AD 5 Occipital Ctx                | 45.1      |
| Control 4 Hippo                  | 13.3      | AD 5 Occipital Ctx                | 0.0       |
| Control (Path) 3<br>Hippo        | 0.0       | Control 1 Occipital<br>Ctx        | 4.5       |
| AD 1 Temporal Ctx                | 25.0      | Control 2 Occipital<br>Ctx        | 58.2      |
| AD 2 Temporal Ctx                | 39.2      | Control 3 Occipital<br>Ctx        | 18.2      |
| AD 3 Temporal Ctx                | 7.7       | Control 4 Occipital<br>Ctx        | 7.3       |
| AD 4 Temporal Ctx                | 0.2       | Control (Path) 1<br>Occipital Ctx | 92.7      |
| AD 5 Inf Temporal<br>Ctx         | 76.8      | Control (Path) 2<br>Occipital Ctx | 0.0       |
| AD 5 Sup Temporal<br>Ctx         | 40.6      | Control (Path) 3<br>Occipital Ctx | 3.4       |
| AD 6 Inf Temporal<br>Ctx         | 49.7      | Control (Path) 4<br>Occipital Ctx | 16.7      |
| AD 6 Sup Temporal<br>Ctx         | 57.4      | Control 1 Parietal Ctx            | 7.1       |
| Control 1 Temporal<br>Ctx        | 9.2       | Control 2 Parietal Ctx            | 41.8      |
| Control 2 Temporal<br>Ctx        | 40.9      | Control 3 Parietal Ctx            | 0.0       |
| Control 3 Temporal<br>Ctx        | 20.6      | Control (Path) 1<br>Parietal Ctx  | 97.9      |
| Control 3 Temporal<br>Ctx        | 10.7      | Control (Path) 2<br>Parietal Ctx  | 29.5      |
| Control (Path) 1<br>Temporal Ctx | 97.3      | Control (Path) 3<br>Parietal Ctx  | 4.4       |
| Control (Path) 2<br>Temporal Ctx | 52.9      | Control (Path) 4<br>Parietal Ctx  | 68.3      |

Table 33. Panel 1.3D

| Tissue Name            | Rel. Exp.(%) Ag2304,<br>Run 159131830 | Tissue Name     | Rel. Exp.(%) Ag2304,<br>Run 159131830 |
|------------------------|---------------------------------------|-----------------|---------------------------------------|
| Liver adenocarcinoma   | 6.0                                   | Kidney (fetal)  | 8.5                                   |
| Pancreas               | 1.7                                   | Renal ca. 786-0 | 7.6                                   |
| Pancreatic ca. CAPAN 2 | 2.4                                   | Renal ca. A498  | 13.2                                  |

|                          |       |                                   |      |
|--------------------------|-------|-----------------------------------|------|
| Adrenal gland            | 14.9  | Renal ca. RXF 393                 | 3.2  |
| Thyroid                  | 6.5   | Renal ca. ACHN                    | 3.1  |
| Salivary gland           | 2.3   | Renal ca. UO-31                   | 8.3  |
| Pituitary gland          | 13.4  | Renal ca. TK-10                   | 3.5  |
| Brain (fetal)            | 7.7   | Liver                             | 2.8  |
| Brain (whole)            | 13.5  | Liver (fetal)                     | 5.8  |
| Brain (amygdala)         | 15.5  | Liver ca. (hepatoblast)<br>HepG2  | 7.3  |
| Brain (cerebellum)       | 4.6   | Lung                              | 19.9 |
| Brain (hippocampus)      | 100.0 | Lung (fetal)                      | 9.9  |
| Brain (substantia nigra) | 2.8   | Lung ca. (small cell)<br>LX-1     | 5.4  |
| Brain (thalamus)         | 10.0  | Lung ca. (small cell)<br>NCI-H69  | 12.3 |
| Cerebral Cortex          | 25.0  | Lung ca. (s.cell var.)<br>SHP-77  | 12.1 |
| Spinal cord              | 4.0   | Lung ca. (large<br>cell)NCI-H460  | 3.8  |
| glio/astro U87-MG        | 21.9  | Lung ca. (non-sm. cell)<br>A549   | 5.9  |
| glio/astro U-118-MG      | 40.9  | Lung ca. (non-s.cell)<br>NCI-H23  | 13.6 |
| astrocytoma SW1783       | 9.2   | Lung ca. (non-s.cell)<br>HOP-62   | 7.0  |
| neuro*; met SK-N-AS      | 65.5  | Lung ca. (non-s.cl)<br>NCI-H522   | 3.4  |
| astrocytoma SF-539       | 9.8   | Lung ca. (squam.) SW<br>900       | 6.6  |
| astrocytoma SNB-75       | 11.9  | Lung ca. (squam.)<br>NCI-H596     | 1.7  |
| glioma SNB-19            | 9.6   | Mammary gland                     | 18.4 |
| glioma U251              | 6.0   | Breast ca.* (pl.ef)<br>MCF-7      | 6.3  |
| glioma SF-295            | 6.5   | Breast ca.* (pl.ef)<br>MDA-MB-231 | 34.6 |
| Heart (Fetal)            | 0.6   | Breast ca.* (pl. ef)<br>T47D      | 5.1  |
| Heart                    | 2.3   | Breast ca. BT-549                 | 20.2 |
| Skeletal muscle (Fetal)  | 11.4  | Breast ca. MDA-N                  | 5.7  |
| Skeletal muscle          | 8.5   | Ovary                             | 5.6  |
| Bone marrow              | 8.5   | Ovarian ca. OVCAR-3               | 7.7  |
| Thymus                   | 7.4   | Ovarian ca. OVCAR-4               | 0.7  |
| Spleen                   | 12.0  | Ovarian ca. OVCAR-5               | 16.7 |
| Lymph node               | 6.3   | Ovarian ca. OVCAR-8               | 8.6  |
| Colorectal               | 4.6   | Ovarian ca. IGROV-1               | 2.4  |
| Stomach                  | 8.5   | Ovarian ca. (ascites)<br>SK-OV-3  | 15.5 |
| Small intestine          | 9.2   | Uterus                            | 6.4  |

|                                    |      |                                  |      |
|------------------------------------|------|----------------------------------|------|
| Colon ca. SW480                    | 8.0  | Placenta                         | 8.1  |
| Colon ca.* SW620<br>(SW480 met)    | 5.3  | Prostate                         | 3.4  |
| Colon ca. HT29                     | 2.6  | Prostate ca.* (bone<br>met) PC-3 | 5.9  |
| Colon ca. HCT-116                  | 7.4  | Testis                           | 18.6 |
| Colon ca. CaCo-2                   | 7.4  | Melanoma Hs688(A).T              | 4.5  |
| CC Well to Mod Diff<br>(ODO3866)   | 9.2  | Melanoma* (met)<br>Hs688(B).T    | 2.5  |
| Colon ca. HCC-2998                 | 8.6  | Melanoma UACC-62                 | 1.6  |
| Gastric ca. (liver met)<br>NCI-N87 | 30.8 | Melanoma M14                     | 0.6  |
| Bladder                            | 2.7  | Melanoma LOX IMVI                | 4.0  |
| Trachea                            | 12.0 | Melanoma* (met) SK-<br>MEL-5     | 2.3  |
| Kidney                             | 3.5  | Adipose                          | 7.5  |

Table 34. Panel 2D

| Tissue Name                                      | Rel. Exp.(%) Ag2304,<br>Run 159134494 | Tissue Name                        | Rel. Exp.(%) Ag2304,<br>Run 159134494 |
|--------------------------------------------------|---------------------------------------|------------------------------------|---------------------------------------|
| Normal Colon                                     | 82.9                                  | Kidney Margin<br>8120608           | 5.5                                   |
| CC Well to Mod Diff<br>(ODO3866)                 | 21.3                                  | Kidney Cancer 8120613              | 17.9                                  |
| CC Margin (ODO3866)                              | 14.6                                  | Kidney Margin<br>8120614           | 13.1                                  |
| CC Gr.2 rectosigmoid<br>(ODO3868)                | 10.9                                  | Kidney Cancer 9010320              | 24.7                                  |
| CC Margin (ODO3868)                              | 9.9                                   | Kidney Margin<br>9010321           | 19.3                                  |
| CC Mod Diff (ODO3920)                            | 21.5                                  | Normal Uterus                      | 17.6                                  |
| CC Margin (ODO3920)                              | 27.4                                  | Uterine Cancer 064011              | 52.5                                  |
| CC Gr.2 ascend colon<br>(ODO3921)                | 45.1                                  | Normal Thyroid                     | 22.7                                  |
| CC Margin (ODO3921)                              | 15.8                                  | Thyroid Cancer                     | 36.1                                  |
| CC from Partial<br>Hepatectomy (ODO4309)<br>Mets | 37.9                                  | Thyroid Cancer<br>A302152          | 18.2                                  |
| Liver Margin (ODO4309)                           | 28.9                                  | Thyroid Margin<br>A302153          | 30.1                                  |
| Colon mets to lung<br>(OD04451-01)               | 23.2                                  | Normal Breast                      | 49.7                                  |
| Lung Margin (OD04451-02)                         | 24.1                                  | Breast Cancer                      | 28.5                                  |
| Normal Prostate 6546-1                           | 18.4                                  | Breast Cancer<br>(OD04590-01)      | 51.8                                  |
| Prostate Cancer (OD04410)                        | 59.9                                  | Breast Cancer Mets<br>(OD04590-03) | 64.6                                  |
| Prostate Margin (OD04410)                        | 67.4                                  | Breast Cancer<br>Metastasis        | 47.6                                  |

|                                       |       |                                      |      |
|---------------------------------------|-------|--------------------------------------|------|
| Prostate Cancer (OD04720-01)          | 46.7  | Breast Cancer                        | 26.2 |
| Prostate Margin (OD04720-02)          | 93.3  | Breast Cancer                        | 28.9 |
| Normal Lung                           | 100.0 | Breast Cancer 9100266                | 20.2 |
| Lung Met to Muscle (ODO4286)          | 41.2  | Breast Margin 9100265                | 16.7 |
| Muscle Margin (ODO4286)               | 47.6  | Breast Cancer A209073                | 38.2 |
| Lung Malignant Cancer (OD03126)       | 31.9  | Breast Margin A2090734               | 44.8 |
| Lung Margin (OD03126)                 | 64.2  | Normal Liver                         | 23.2 |
| Lung Cancer (OD04404)                 | 58.6  | Liver Cancer                         | 23.3 |
| Lung Margin (OD04404)                 | 38.2  | Liver Cancer 1025                    | 10.5 |
| Lung Cancer (OD04565)                 | 15.8  | Liver Cancer 1026                    | 6.7  |
| Lung Margin (OD04565)                 | 26.4  | Liver Cancer 6004-T                  | 14.1 |
| Lung Cancer (OD04237-01)              | 37.6  | Liver Tissue 6004-N                  | 9.5  |
| Lung Margin (OD04237-02)              | 48.0  | Liver Cancer 6005-T                  | 6.7  |
| Ocular Mel Met to Liver (ODO4310)     | 14.9  | Liver Tissue 6005-N                  | 6.7  |
| Liver Margin (ODO4310)                | 13.5  | Normal Bladder                       | 49.0 |
| Melanoma Metastasis                   | 36.6  | Bladder Cancer                       | 5.7  |
| Lung Margin (OD04321)                 | 50.3  | Bladder Cancer                       | 32.5 |
| Normal Kidney                         | 84.7  | Bladder Cancer (OD04718-01)          | 52.1 |
| Kidney Ca, Nuclear grade 2 (OD04338)  | 65.1  | Bladder Normal Adjacent (OD04718-03) | 63.7 |
| Kidney Margin (OD04338)               | 46.3  | Normal Ovary                         | 6.0  |
| Kidney Ca Nuclear grade 1/2 (OD04339) | 33.4  | Ovarian Cancer                       | 63.3 |
| Kidney Margin (OD04339)               | 77.9  | Ovarian Cancer (OD04768-07)          | 43.8 |
| Kidney Ca, Clear cell type (OD04340)  | 71.7  | Ovary Margin (OD04768-08)            | 14.6 |
| Kidney Margin (OD04340)               | 57.0  | Normal Stomach                       | 30.4 |
| Kidney Ca, Nuclear grade 3 (OD04348)  | 17.2  | Gastric Cancer 9060358               | 10.4 |
| Kidney Margin (OD04348)               | 28.9  | Stomach Margin 9060359               | 12.9 |
| Kidney Cancer (OD04622-01)            | 21.9  | Gastric Cancer 9060395               | 56.3 |
| Kidney Margin (OD04622-03)            | 4.3   | Stomach Margin 9060394               | 30.4 |
| Kidney Cancer (OD04450-01)            | 29.5  | Gastric Cancer 9060397               | 33.2 |
| Kidney Margin (OD04450-03)            | 36.9  | Stomach Margin 9060396               | 8.9  |
| Kidney Cancer 8120607                 | 3.4   | Gastric Cancer 064005                | 53.6 |

Table 35. Panel 4D

| Tissue Name                        | Rel. Exp.(%)<br>Ag2304, Run<br>159131012 | Tissue Name                                    | Rel. Exp.(%)<br>Ag2304, Run<br>159131012 |
|------------------------------------|------------------------------------------|------------------------------------------------|------------------------------------------|
| Secondary Th1 act                  | 32.5                                     | HUVEC IL-1beta                                 | 5.8                                      |
| Secondary Th2 act                  | 46.7                                     | HUVEC IFN gamma                                | 19.6                                     |
| Secondary Tr1 act                  | 47.0                                     | HUVEC TNF alpha + IFN<br>gamma                 | 12.2                                     |
| Secondary Th1 rest                 | 14.5                                     | HUVEC TNF alpha + IL4                          | 9.8                                      |
| Secondary Th2 rest                 | 27.0                                     | HUVEC IL-11                                    | 8.8                                      |
| Secondary Tr1 rest                 | 23.5                                     | Lung Microvascular EC none                     | 5.8                                      |
| Primary Th1 act                    | 44.1                                     | Lung Microvascular EC<br>TNFalpha + IL-1beta   | 12.8                                     |
| Primary Th2 act                    | 39.2                                     | Microvascular Dermal EC<br>none                | 20.6                                     |
| Primary Tr1 act                    | 49.3                                     | Microvascular Dermal EC<br>TNFalpha + IL-1beta | 16.0                                     |
| Primary Th1 rest                   | 95.3                                     | Bronchial epithelium<br>TNFalpha + IL1beta     | 14.2                                     |
| Primary Th2 rest                   | 54.7                                     | Small airway epithelium<br>none                | 7.9                                      |
| Primary Tr1 rest                   | 29.5                                     | Small airway epithelium<br>TNFalpha + IL-1beta | 38.4                                     |
| CD45RA CD4<br>lymphocyte act       | 21.5                                     | Coronary artery SMC rest                       | 25.3                                     |
| CD45RO CD4<br>lymphocyte act       | 37.1                                     | Coronary artery SMC<br>TNFalpha + IL-1beta     | 12.7                                     |
| CD8 lymphocyte act                 | 20.9                                     | Astrocytes rest                                | 23.0                                     |
| Secondary CD8<br>lymphocyte rest   | 29.1                                     | Astrocytes TNFalpha + IL-<br>1beta             | 23.7                                     |
| Secondary CD8<br>lymphocyte act    | 22.7                                     | KU-812 (Basophil) rest                         | 4.6                                      |
| CD4 lymphocyte none                | 26.6                                     | KU-812 (Basophil)<br>PMA/ionomycin             | 11.0                                     |
| 2ry Th1/Th2/Tr1_anti-<br>CD95 CH11 | 34.2                                     | CCD1106 (Keratinocytes)<br>none                | 15.8                                     |
| LAK cells rest                     | 41.5                                     | CCD1106 (Keratinocytes)<br>TNFalpha + IL-1beta | 5.1                                      |
| LAK cells IL-2                     | 33.2                                     | Liver cirrhosis                                | 2.8                                      |
| LAK cells IL-2+IL-12               | 22.8                                     | Lupus kidney                                   | 4.3                                      |
| LAK cells IL-2+IFN<br>gamma        | 36.9                                     | NCI-H292 none                                  | 34.9                                     |
| LAK cells IL-2+ IL-18              | 38.4                                     | NCI-H292 IL-4                                  | 38.4                                     |
| LAK cells<br>PMA/ionomycin         | 34.2                                     | NCI-H292 IL-9                                  | 39.2                                     |
| NK Cells IL-2 rest                 | 26.6                                     | NCI-H292 IL-13                                 | 21.2                                     |
| Two Way MLR 3 day                  | 53.2                                     | NCI-H292 IFN gamma                             | 20.9                                     |
| Two Way MLR 5 day                  | 26.2                                     | HPAEC none                                     | 11.3                                     |

|                              |       |                                       |      |
|------------------------------|-------|---------------------------------------|------|
| Two Way MLR 7 day            | 13.3  | HPAEC TNF alpha + IL-1 beta           | 11.7 |
| PBMC rest                    | 14.5  | Lung fibroblast none                  | 18.9 |
| PBMC PWM                     | 83.5  | Lung fibroblast TNF alpha + IL-1 beta | 22.8 |
| PBMC PHA-L                   | 31.9  | Lung fibroblast IL-4                  | 25.9 |
| Ramos (B cell) none          | 11.4  | Lung fibroblast IL-9                  | 13.4 |
| Ramos (B cell) ionomycin     | 34.4  | Lung fibroblast IL-13                 | 18.9 |
| B lymphocytes PWM            | 60.3  | Lung fibroblast IFN gamma             | 46.0 |
| B lymphocytes CD40L and IL-4 | 16.6  | Dermal fibroblast CCD1070 rest        | 47.0 |
| EOL-1 dbcAMP                 | 34.2  | Dermal fibroblast CCD1070 TNF alpha   | 83.5 |
| EOL-1 dbcAMP PMA/ionomycin   | 100.0 | Dermal fibroblast CCD1070 IL-1 beta   | 23.7 |
| Dendritic cells none         | 13.7  | Dermal fibroblast IFN gamma           | 18.3 |
| Dendritic cells LPS          | 16.5  | Dermal fibroblast IL-4                | 25.2 |
| Dendritic cells anti-CD40    | 6.3   | IBD Colitis 2                         | 2.3  |
| Monocytes rest               | 23.5  | IBD Crohn's                           | 4.3  |
| Monocytes LPS                | 84.1  | Colon                                 | 24.5 |
| Macrophages rest             | 23.3  | Lung                                  | 23.7 |
| Macrophages LPS              | 31.4  | Thymus                                | 39.0 |
| HUVEC none                   | 15.0  | Kidney                                | 44.4 |
| HUVEC starved                | 30.6  |                                       |      |

**CNS\_neurodegeneration\_v1.0 Summary:** Ag2304 Expression of the NOV3 gene in this panel is ubiquitous. While this gene does not show differential expression between Alzheimer's diseased brains and control brains, this panel confirms the expression of this gene in the brains of an independent group of patients. See Panel 1.3d for utility of this gene in the central nervous system.

**Panel 1.3D Summary:** Ag2304 The NOV3 gene, a homolog of the Drosophila pecanex gene, is widely expressed across the samples in this panel, with highest expression in the hippocampus (CT=28.6). In addition, this gene is expressed at moderate to high levels in all CNS regions examined. Expression of this gene in both the mother and developing embryo is critical for normal CNS development. Furthermore, expression of this protein appears to be involved in stem cell fate determination, where removal of this protein increases neural precursor cells. Therefore, downregulation of this gene could be used in neural stem cell research and therapy to control the fate of stem cells and increasing the resulting numbers of post-mitotic neurons.



The NOV3 gene is modestly expressed in a wide variety of metabolic tissues including adipose, adrenal, pancreas, thyroid, pituitary, heart, adult and fetal skeletal muscle, and adult and fetal liver. This widespread expression in tissues with metabolic function suggests that the NOV3 gene product may be important for the pathogenesis, diagnosis, and/or treatment of metabolic disease in any or all of these tissues, including obesity and diabetes.

References:

1. LaBonne SG, Furst A. Differentiation in vitro of neural precursor cells from normal and Pecanex mutant *Drosophila* embryos. *J Neurogenet* 1989 May;5(2):99-104

Early gastrula embryos, lacking both maternally and zygotically expressed activity of the neurogenic pecanex locus, are shown to contain a greater than wild-type number of stably determined neural precursor cells which can differentiate into neurons in culture.

2. LaBonne SG, Sunitha I, Mahowald AP. Molecular genetics of pecanex, a maternal-effect neurogenic locus of *Drosophila melanogaster* that potentially encodes a large transmembrane protein. *Dev Biol* 1989 Nov;136(1):1-16

In the absence of maternal expression of the pecanex gene, the embryo develops severe hyperneuralization similar to that characteristic of Notch mutant embryos. We have extended a previous molecular analysis of the chromosomal interval that encompasses pecanex by using additional deficiencies to localize the locus on the molecular map. RNA blot analysis shows that the locus encodes a rare 9-kb transcript as well as minor transcripts of 3.7 and 2.3 kb. The temporal expression of these transcripts is appropriate for a neurogenic locus. Phenocopies of the mutant phenotype have been produced following microinjection of antisense RNA corresponding to a portion of the pecanex transcripts. Conceptual translation of a partial coding sequence compiled from cDNA and genomic clones indicates that the pecanex locus potentially encodes a large, membrane-spanning protein.

**Panel 2D Summary:** Ag2304 The expression of this gene appears to be highest in a sample derived from normal lung tissue. Thus, the expression of this gene could be used to distinguish normal lung tissue from other tissues in the panel. Of note is the difference in expression between samples derived from ovarian cancer and normal adjacent tissue. This difference in levels of expression is also notable in samples derived from gastric cancer when compared to their normal counterparts. Thus, the expression of this gene could be used to distinguish ovarian or gastric cancer from their normal adjacent tissues. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be of use in the treatment of ovarian or gastric cancer.

**Panel 4D Summary:** Ag 2304 This NOV3 transcript is detected ubiquitously throughout this panel, with highest expression of this transcript in activated eosinophils (CT=28.1). This indicates an up-regulation of this transcript in these cells upon activation. Eosinophils contribute to the pathology of several atopic diseases such as asthma, atopic dermatitis, and rhinitis. Therefore, modulation of the activity or activation of the protein encoded by the NOV3 gene may be beneficial for the treatment of those diseases. The NOV3 gene is also highly expressed in effector T cells, activated monocytes and dermal fibroblasts upon treatment with TNF- $\alpha$  and IL-1 $\beta$ . Modulation of the expression of this transcript, which encodes for a Pecanex like molecule, could be beneficial in the treatment of inflammatory diseases associated with T cell activation as well as eosinophil activation including atopic diseases and autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease and skin inflammation.

#### NOV4

Expression of gene NOV4 was assessed using the primer-probe set Ag2428, described in Table 36. Results of the RTQ-PCR runs are shown in Tables 37, 38, 39 and 40.

**Table 36. Probe Name Ag2428**

| Primers | Sequences                                         | Length | Start Position | SEQ ID NO: |
|---------|---------------------------------------------------|--------|----------------|------------|
| Forward | 5'-gagccagggctgctgtata-3'                         | 19     | 1419           | 154        |
| Probe   | TET-5'-<br>cctctcaggaacatgctaccaaatt-3'-<br>TAMRA | 26     | 1439           | 155        |
| Reverse | 5'-tagattgagggcagcagtc-3'                         | 20     | 1476           | 156        |

**Table 37. CNS\_neurodegeneration\_v1.0**

| Tissue Name            | Rel. Exp.(%) Ag2428, Run 206271177 | Tissue Name                   | Rel. Exp.(%) Ag2428, Run 206271177 |
|------------------------|------------------------------------|-------------------------------|------------------------------------|
| AD 1 Hippo             | 7.9                                | Control (Path) 3 Temporal Ctx | 3.5                                |
| AD 2 Hippo             | 22.2                               | Control (Path) 4 Temporal Ctx | 40.3                               |
| AD 3 Hippo             | 12.8                               | AD 1 Occipital Ctx            | 18.3                               |
| AD 4 Hippo             | 5.1                                | AD 2 Occipital Ctx (Missing)  | 0.0                                |
| AD 5 Hippo             | 100.0                              | AD 3 Occipital Ctx            | 7.0                                |
| AD 6 Hippo             | 32.5                               | AD 4 Occipital Ctx            | 20.3                               |
| Control 2 Hippo        | 10.9                               | AD 5 Occipital Ctx            | 25.0                               |
| Control 4 Hippo        | 17.0                               | AD 5 Occipital Ctx            | 16.5                               |
| Control (Path) 3 Hippo | 6.7                                | Control 1 Occipital Ctx       | 6.0                                |

|                               |      |                                |      |
|-------------------------------|------|--------------------------------|------|
| AD 1 Temporal Ctx             | 16.6 | Control 2 Occipital Ctx        | 21.0 |
| AD 2 Temporal Ctx             | 23.2 | Control 3 Occipital Ctx        | 23.2 |
| AD 3 Temporal Ctx             | 9.4  | Control 4 Occipital Ctx        | 6.0  |
| AD 4 Temporal Ctx             | 25.9 | Control (Path) 1 Occipital Ctx | 50.3 |
| AD 5 Inf Temporal Ctx         | 40.1 | Control (Path) 2 Occipital Ctx | 13.2 |
| AD 5 Sup Temporal Ctx         | 33.7 | Control (Path) 3 Occipital Ctx | 1.1  |
| AD 6 Inf Temporal Ctx         | 35.6 | Control (Path) 4 Occipital Ctx | 30.4 |
| AD 6 Sup Temporal Ctx         | 48.3 | Control 1 Parietal Ctx         | 12.4 |
| Control 1 Temporal Ctx        | 8.0  | Control 2 Parietal Ctx         | 46.0 |
| Control 2 Temporal Ctx        | 8.5  | Control 3 Parietal Ctx         | 23.7 |
| Control 3 Temporal Ctx        | 14.7 | Control (Path) 1 Parietal Ctx  | 38.7 |
| Control 3 Temporal Ctx        | 11.3 | Control (Path) 2 Parietal Ctx  | 20.4 |
| Control (Path) 1 Temporal Ctx | 37.6 | Control (Path) 3 Parietal Ctx  | 5.4  |
| Control (Path) 2 Temporal Ctx | 34.9 | Control (Path) 4 Parietal Ctx  | 43.2 |

Table 38. Panel 1.3D

| Tissue Name              | Rel. Exp.(%) Ag2428, Run 159361380 | Tissue Name                   | Rel. Exp.(%) Ag2428, Run 159361380 |
|--------------------------|------------------------------------|-------------------------------|------------------------------------|
| Liver adenocarcinoma     | 10.7                               | Kidney (fetal)                | 7.1                                |
| Pancreas                 | 2.4                                | Renal ca. 786-0               | 6.6                                |
| Pancreatic ca. CAPAN 2   | 6.0                                | Renal ca. A498                | 18.8                               |
| Adrenal gland            | 5.3                                | Renal ca. RXF 393             | 3.8                                |
| Thyroid                  | 2.5                                | Renal ca. ACHN                | 1.1                                |
| Salivary gland           | 3.7                                | Renal ca. UO-31               | 4.5                                |
| Pituitary gland          | 7.2                                | Renal ca. TK-10               | 5.1                                |
| Brain (fetal)            | 5.2                                | Liver                         | 1.9                                |
| Brain (whole)            | 5.1                                | Liver (fetal)                 | 11.4                               |
| Brain (amygdala)         | 5.1                                | Liver ca. (hepatoblast) HepG2 | 8.0                                |
| Brain (cerebellum)       | 2.7                                | Lung                          | 8.5                                |
| Brain (hippocampus)      | 17.6                               | Lung (fetal)                  | 4.7                                |
| Brain (substantia nigra) | 1.8                                | Lung ca. (small cell) LX-1    | 7.5                                |
| Brain (thalamus)         | 4.9                                | Lung ca. (small cell) NCI-H69 | 11.9                               |

|                                    |       |                                   |      |
|------------------------------------|-------|-----------------------------------|------|
| Cerebral Cortex                    | 2.8   | Lung ca. (s.cell var.)<br>SHP-77  | 25.2 |
| Spinal cord                        | 3.8   | Lung ca. (large<br>cell)NCI-H460  | 8.8  |
| glio/astro U87-MG                  | 12.9  | Lung ca. (non-sm. cell)<br>A549   | 8.3  |
| glio/astro U-118-MG                | 39.5  | Lung ca. (non-s.cell)<br>NCI-H23  | 18.3 |
| astrocytoma SW1783                 | 5.4   | Lung ca. (non-s.cell)<br>HOP-62   | 6.6  |
| neuro*; met SK-N-AS                | 100.0 | Lung ca. (non-s.cl)<br>NCI-H522   | 8.4  |
| astrocytoma SF-539                 | 7.6   | Lung ca. (squam.) SW<br>900       | 9.7  |
| astrocytoma SNB-75                 | 19.8  | Lung ca. (squam.)<br>NCI-H596     | 5.4  |
| glioma SNB-19                      | 12.0  | Mammary gland                     | 5.9  |
| glioma U251                        | 11.3  | Breast ca.* (pl.ef)<br>MCF-7      | 10.9 |
| glioma SF-295                      | 7.4   | Breast ca.* (pl.ef)<br>MDA-MB-231 | 66.0 |
| Heart (Fetal)                      | 2.0   | Breast ca.* (pl. ef)<br>T47D      | 9.6  |
| Heart                              | 5.1   | Breast ca. BT-549                 | 34.4 |
| Skeletal muscle (Fetal)            | 8.5   | Breast ca. MDA-N                  | 17.7 |
| Skeletal muscle                    | 1.2   | Ovary                             | 2.1  |
| Bone marrow                        | 17.8  | Ovarian ca. OVCAR-3               | 10.8 |
| Thymus                             | 5.6   | Ovarian ca. OVCAR-4               | 0.6  |
| Spleen                             | 10.1  | Ovarian ca. OVCAR-5               | 5.6  |
| Lymph node                         | 9.2   | Ovarian ca. OVCAR-8               | 10.0 |
| Colorectal                         | 6.9   | Ovarian ca. IGROV-1               | 2.1  |
| Stomach                            | 7.3   | Ovarian ca. (ascites)<br>SK-OV-3  | 15.1 |
| Small intestine                    | 8.1   | Uterus                            | 3.7  |
| Colon ca. SW480                    | 8.5   | Placenta                          | 3.8  |
| Colon ca.* SW620<br>(SW480 met)    | 13.6  | Prostate                          | 6.0  |
| Colon ca. HT29                     | 11.0  | Prostate ca.* (bone<br>met) PC-3  | 5.8  |
| Colon ca. HCT-116                  | 12.9  | Testis                            | 9.3  |
| Colon ca. CaCo-2                   | 12.3  | Melanoma Hs688(A).T               | 2.6  |
| CC Well to Mod Diff<br>(ODO3866)   | 7.6   | Melanoma* (met)<br>Hs688(B).T     | 1.9  |
| Colon ca. HCC-2998                 | 33.0  | Melanoma UACC-62                  | 3.8  |
| Gastric ca. (liver met)<br>NCI-N87 | 25.9  | Melanoma M14                      | 5.8  |
| Bladder                            | 10.2  | Melanoma LOX IMVI                 | 5.2  |
| Trachea                            | 9.0   | Melanoma* (met) SK-               | 10.3 |

|        |     |         |     |
|--------|-----|---------|-----|
|        |     | MEL-5   |     |
| Kidney | 2.5 | Adipose | 6.3 |

Table 39. Panel 2D

| Tissue Name                                      | Rel. Exp.(%) Ag2428,<br>Run 159361727 | Tissue Name                        | Rel. Exp.(%) Ag2428,<br>Run 159361727 |
|--------------------------------------------------|---------------------------------------|------------------------------------|---------------------------------------|
| Normal Colon                                     | 80.1                                  | Kidney Margin<br>8120608           | 2.1                                   |
| CC Well to Mod Diff<br>(ODO3866)                 | 13.7                                  | Kidney Cancer 8120613              | 15.3                                  |
| CC Margin (ODO3866)                              | 7.7                                   | Kidney Margin<br>8120614           | 6.8                                   |
| CC Gr.2 rectosigmoid<br>(ODO3868)                | 25.5                                  | Kidney Cancer 9010320              | 21.3                                  |
| CC Margin (ODO3868)                              | 6.6                                   | Kidney Margin<br>9010321           | 17.4                                  |
| CC Mod Diff (ODO3920)                            | 70.2                                  | Normal Uterus                      | 3.2                                   |
| CC Margin (ODO3920)                              | 30.4                                  | Uterine Cancer 064011              | 21.3                                  |
| CC Gr.2 ascend colon<br>(ODO3921)                | 52.1                                  | Normal Thyroid                     | 9.9                                   |
| CC Margin (ODO3921)                              | 11.1                                  | Thyroid Cancer                     | 5.8                                   |
| CC from Partial<br>Hepatectomy (ODO4309)<br>Mets | 50.7                                  | Thyroid Cancer<br>A302152          | 27.7                                  |
| Liver Margin (ODO4309)                           | 22.7                                  | Thyroid Margin<br>A302153          | 37.6                                  |
| Colon mets to lung<br>(OD04451-01)               | 29.1                                  | Normal Breast                      | 18.7                                  |
| Lung Margin (OD04451-02)                         | 7.0                                   | Breast Cancer                      | 26.4                                  |
| Normal Prostate 6546-1                           | 8.8                                   | Breast Cancer<br>(OD04590-01)      | 75.3                                  |
| Prostate Cancer (OD04410)                        | 49.3                                  | Breast Cancer Mets<br>(OD04590-03) | 87.1                                  |
| Prostate Margin (OD04410)                        | 41.2                                  | Breast Cancer<br>Metastasis        | 48.0                                  |
| Prostate Cancer (OD04720-<br>01)                 | 52.9                                  | Breast Cancer                      | 49.7                                  |
| Prostate Margin (OD04720-<br>02)                 | 59.5                                  | Breast Cancer                      | 36.3                                  |
| Normal Lung                                      | 81.8                                  | Breast Cancer 9100266              | 18.4                                  |
| Lung Met to Muscle<br>(ODO4286)                  | 30.1                                  | Breast Margin 9100265              | 14.9                                  |
| Muscle Margin (ODO4286)                          | 13.9                                  | Breast Cancer A209073              | 55.5                                  |
| Lung Malignant Cancer<br>(OD03126)               | 47.3                                  | Breast Margin<br>A2090734          | 45.1                                  |
| Lung Margin (OD03126)                            | 41.8                                  | Normal Liver                       | 15.8                                  |
| Lung Cancer (OD04404)                            | 28.5                                  | Liver Cancer                       | 14.4                                  |
| Lung Margin (OD04404)                            | 16.7                                  | Liver Cancer 1025                  | 5.7                                   |
| Lung Cancer (OD04565)                            | 28.3                                  | Liver Cancer 1026                  | 5.7                                   |

|                                       |      |                                      |       |
|---------------------------------------|------|--------------------------------------|-------|
| Lung Margin (OD04565)                 | 14.0 | Liver Cancer 6004-T                  | 7.9   |
| Lung Cancer (OD04237-01)              | 62.4 | Liver Tissue 6004-N                  | 8.1   |
| Lung Margin (OD04237-02)              | 28.3 | Liver Cancer 6005-T                  | 5.2   |
| Ocular Mel Met to Liver (ODO4310)     | 23.5 | Liver Tissue 6005-N                  | 0.8   |
| Liver Margin (ODO4310)                | 11.3 | Normal Bladder                       | 81.8  |
| Melanoma Metastasis                   | 40.9 | Bladder Cancer                       | 9.2   |
| Lung Margin (OD04321)                 | 26.2 | Bladder Cancer                       | 62.0  |
| Normal Kidney                         | 54.3 | Bladder Cancer (OD04718-01)          | 32.8  |
| Kidney Ca, Nuclear grade 2 (OD04338)  | 40.1 | Bladder Normal Adjacent (OD04718-03) | 24.8  |
| Kidney Margin (OD04338)               | 45.7 | Normal Ovary                         | 0.8   |
| Kidney Ca Nuclear grade 1/2 (OD04339) | 82.9 | Ovarian Cancer                       | 51.8  |
| Kidney Margin (OD04339)               | 45.4 | Ovarian Cancer (OD04768-07)          | 86.5  |
| Kidney Ca, Clear cell type (OD04340)  | 49.3 | Ovary Margin (OD04768-08)            | 8.5   |
| Kidney Margin (OD04340)               | 48.0 | Normal Stomach                       | 20.7  |
| Kidney Ca, Nuclear grade 3 (OD04348)  | 24.3 | Gastric Cancer 9060358               | 6.9   |
| Kidney Margin (OD04348)               | 40.3 | Stomach Margin 9060359               | 13.1  |
| Kidney Cancer (OD04622-01)            | 10.7 | Gastric Cancer 9060395               | 23.5  |
| Kidney Margin (OD04622-03)            | 3.1  | Stomach Margin 9060394               | 18.9  |
| Kidney Cancer (OD04450-01)            | 24.8 | Gastric Cancer 9060397               | 39.8  |
| Kidney Margin (OD04450-03)            | 25.5 | Stomach Margin 9060396               | 7.1   |
| Kidney Cancer 8120607                 | 2.7  | Gastric Cancer 064005                | 100.0 |

Table 40. Panel 4D

| Tissue Name        | Rel. Exp.(%)<br>Ag2428, Run<br>159362614 | Tissue Name                               | Rel. Exp.(%)<br>Ag2428, Run<br>159362614 |
|--------------------|------------------------------------------|-------------------------------------------|------------------------------------------|
| Secondary Th1 act  | 26.8                                     | HUVEC IL-1beta                            | 9.2                                      |
| Secondary Th2 act  | 34.6                                     | HUVEC IFN gamma                           | 14.1                                     |
| Secondary Tr1 act  | 37.6                                     | HUVEC TNF alpha + IFN gamma               | 8.4                                      |
| Secondary Th1 rest | 10.7                                     | HUVEC TNF alpha + IL4                     | 12.0                                     |
| Secondary Th2 rest | 13.6                                     | HUVEC IL-11                               | 7.9                                      |
| Secondary Tr1 rest | 16.7                                     | Lung Microvascular EC none                | 10.9                                     |
| Primary Th1 act    | 36.9                                     | Lung Microvascular EC TNFalpha + IL-1beta | 9.9                                      |
| Primary Th2 act    | 48.3                                     | Microvascular Dermal EC                   | 17.0                                     |

|                                    |       |                                                 |      |
|------------------------------------|-------|-------------------------------------------------|------|
|                                    |       | none                                            |      |
| Primary Tr1 act                    | 50.7  | Microsvascular Dermal EC<br>TNFalpha + IL-1beta | 10.6 |
| Primary Th1 rest                   | 74.2  | Bronchial epithelium<br>TNFalpha + IL1beta      | 9.8  |
| Primary Th2 rest                   | 41.5  | Small airway epithelium<br>none                 | 3.6  |
| Primary Tr1 rest                   | 28.9  | Small airway epithelium<br>TNFalpha + IL-1beta  | 38.7 |
| CD45RA CD4<br>lymphocyte act       | 22.7  | Coronary artery SMC rest                        | 9.9  |
| CD45RO CD4<br>lymphocyte act       | 31.0  | Coronary artery SMC<br>TNFalpha + IL-1beta      | 4.2  |
| CD8 lymphocyte act                 | 15.9  | Astrocytes rest                                 | 5.9  |
| Secondary CD8<br>lymphocyte rest   | 19.6  | Astrocytes TNFalpha + IL-<br>1beta              | 5.8  |
| Secondary CD8<br>lymphocyte act    | 17.9  | KU-812 (Basophil) rest                          | 8.7  |
| CD4 lymphocyte none                | 11.6  | KU-812 (Basophil)<br>PMA/ionomycin              | 31.6 |
| 2ry Th1/Th2/Tr1_anti-<br>CD95 CH11 | 18.8  | CCD1106 (Keratinocytes)<br>none                 | 12.3 |
| LAK cells rest                     | 20.4  | CCD1106 (Keratinocytes)<br>TNFalpha + IL-1beta  | 8.4  |
| LAK cells IL-2                     | 27.7  | Liver cirrhosis                                 | 4.7  |
| LAK cells IL-2+IL-12               | 20.0  | Lupus kidney                                    | 2.1  |
| LAK cells IL-2+IFN<br>gamma        | 38.4  | NCI-H292 none                                   | 25.5 |
| LAK cells IL-2+ IL-18              | 43.5  | NCI-H292 IL-4                                   | 37.1 |
| LAK cells<br>PMA/ionomycin         | 13.6  | NCI-H292 IL-9                                   | 36.9 |
| NK Cells IL-2 rest                 | 21.2  | NCI-H292 IL-13                                  | 15.6 |
| Two Way MLR 3 day                  | 23.8  | NCI-H292 IFN gamma                              | 14.1 |
| Two Way MLR 5 day                  | 11.3  | HPAEC none                                      | 11.1 |
| Two Way MLR 7 day                  | 11.3  | HPAEC TNF alpha + IL-1<br>beta                  | 13.5 |
| PBMC rest                          | 7.9   | Lung fibroblast none                            | 10.7 |
| PBMC PWM                           | 60.3  | Lung fibroblast TNF alpha +<br>IL-1 beta        | 5.0  |
| PBMC PHA-L                         | 23.5  | Lung fibroblast IL-4                            | 18.6 |
| Ramos (B cell) none                | 23.5  | Lung fibroblast IL-9                            | 13.0 |
| Ramos (B cell)<br>ionomycin        | 80.7  | Lung fibroblast IL-13                           | 11.0 |
| B lymphocytes PWM                  | 100.0 | Lung fibroblast IFN gamma                       | 13.6 |
| B lymphocytes CD40L<br>and IL-4    | 44.8  | Dermal fibroblast CCD1070<br>rest               | 27.9 |
| EOL-1 dbcAMP                       | 11.0  | Dermal fibroblast CCD1070<br>TNF alpha          | 82.9 |

|                               |      |                                        |      |
|-------------------------------|------|----------------------------------------|------|
| EOL-1 dbcAMP<br>PMA/ionomycin | 19.6 | Dermal fibroblast CCD1070<br>IL-1 beta | 15.0 |
| Dendritic cells none          | 9.5  | Dermal fibroblast IFN<br>gamma         | 10.1 |
| Dendritic cells LPS           | 7.6  | Dermal fibroblast IL-4                 | 11.9 |
| Dendritic cells anti-<br>CD40 | 5.5  | IBD Colitis 2                          | 2.8  |
| Monocytes rest                | 15.8 | IBD Crohn's                            | 2.2  |
| Monocytes LPS                 | 11.0 | Colon                                  | 11.0 |
| Macrophages rest              | 9.3  | Lung                                   | 5.3  |
| Macrophages LPS               | 5.2  | Thymus                                 | 18.6 |
| HUVEC none                    | 12.2 | Kidney                                 | 41.8 |
| HUVEC starved                 | 33.0 |                                        |      |

**CNS\_neurodegeneration\_v1.0 Summary:** Ag2428 While results from this experiment show that this gene is not differentially expressed in the Alzheimer's diseased brain, this panel confirms the expression of this gene at moderate levels in the CNS in an independent group of patients. Please see Panel 1.3D for a discussion of utility of this gene in the central nervous system.

**Panel 1.3D Summary:** Ag2428 The NOV4 gene is expressed widely across many samples in this panel, with highest expression in a sample derived from a neuroblastoma cell line (CT=29.8). Moreover, there appears to be a cluster of expression associated with breast cancer cell lines. Thus, the expression of this gene could be used to distinguish these samples from others in the panel.

In addition, the NOV4 gene is moderately expressed in a number of metabolic tissues including adipose, adrenal, pituitary, heart, fetal skeletal muscle and fetal liver. Thus, this gene product may be an important small molecule target for the treatment of metabolic disease, including obesity and Type 2 diabetes.

This gene is expressed at low levels in the CNS, and is an an aurora-related kinase. The aurora-related kinases are involved in the control of the cell-cycle, and may be useful in the control of cell fate in neural stem cells. This protein may therefore be of use in stem cell research or therapy.

#### References:

Severson AF, Hamill DR, Carter JC, Schumacher J, Bowerman B. The aurora-related kinase AIR-2 recruits ZEN-4/CeMKLP1 to the mitotic spindle at metaphase and is required for cytokinesis. *Curr Biol* 2000 Oct 5;10(19):1162-71

**BACKGROUND:** The Aurora/Ipl1p-related kinase AIR-2 is required for mitotic chromosome segregation and cytokinesis in early *Caenorhabditis elegans* embryos. Previous



studies have relied on non-conditional mutations or RNA-mediated interference (RNAi) to inactivate AIR-2. It has therefore not been possible to determine whether AIR-2 functions directly in cytokinesis or if the cleavage defect results indirectly from the failure to segregate DNA. One intriguing hypothesis is that AIR-2 acts to localize the mitotic kinesin-like protein ZEN-4 (also known as CeMKLP1), which later functions in cytokinesis. RESULTS: Using conditional alleles, we established that AIR-2 is required at metaphase or early anaphase for normal segregation of chromosomes, localization of ZEN-4, and cytokinesis. ZEN-4 is first required late in cytokinesis, and also functions to maintain cell separation through much of the subsequent interphase. DNA segregation defects alone were not sufficient to disrupt cytokinesis in other mutants, suggesting that AIR-2 acts specifically during cytokinesis through ZEN-4. AIR-2 and ZEN-4 shared similar genetic interactions with the formin homology (FH) protein CYK-1, suggesting that AIR-2 and ZEN-4 function in a single pathway, in parallel to a contractile ring pathway that includes CYK-1. Using in vitro co-immunoprecipitation experiments, we found that AIR-2 and ZEN-4 interact directly. CONCLUSIONS: AIR-2 has two functions during mitosis: one in chromosome segregation, and a second, independent function in cytokinesis through ZEN-4. AIR-2 and ZEN-4 may act in parallel to a second pathway that includes CYK-1.

**Panel 2D Summary:** Ag2428 The expression of this gene is found widely across a number of samples in this panel. It is found to be highest in a sample derived from a gastric cancer. Of note is the association observed between gastric cancer samples, when compared to their normal adjacent samples. This association is also notable in ovarian cancer and breast cancer. Thus, the expression of this gene could be used to distinguish gastric cancer, breast cancer and ovarian cancer from their normal adjacent tissues. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be of benefit in the treatment of gastric, breast or ovarian cancer.

**Panel 4D Summary:** Ag 2428 This transcript is ubiquitously expressed in all cells throughout the panel. However, the highest expression of this transcript is found in B cells upon activation with the B cell mitogen, PWM. Significant expression of this transcript in the activated Ramos B cell line is consistent with this finding. This transcript encodes an aurora-related kinase 1 which belongs to a family of oncogenic mitogenic serine threonine kinases (see reference below). Therefore, modulation of the expression of this transcript by small molecules, may be beneficial for the treatment of diseases associated with hyperproliferation of B cells including B cell lymphomas, hyperglobulinemia and autoimmune disease such as lupus and rheumatoid arthritis. This transcript is also expressed in dermal fibroblasts upon

treatment with TNF- $\alpha$  and IL-1 $\alpha$  in primary Th1 cells suggesting that modulation of this transcript may be important in the treatment of T cell mediated diseases and inflammatory skin diseases.

Reference:

1. J Cell Sci 1999 Nov;112 ( Pt 21):3591-601. Aurora/Ipl1p-related kinases, a new oncogenic family of mitotic serine-threonine kinases. Giet R, Prigent C.  
CNRS UPR41| Universite de Rennes I, Groupe Cycle Cellulaire, Faculte de Medecine, CS 34317, France.

During the past five years, a growing number of serine-threonine kinases highly homologous to the *Saccharomyces cerevisiae* Ipl1p kinase have been isolated in various organisms. A *Drosophila melanogaster* homologue, aurora, was the first to be isolated from a multicellular organism. Since then, several related kinases have been found in mammalian cells. They localise to the mitotic apparatus: in the centrosome, at the poles of the bipolar spindle or in the midbody. The kinases are necessary for completion of mitotic events such as centrosome separation, bipolar spindle assembly and chromosome segregation. Extensive research is now focusing on these proteins because the three human homologues are overexpressed in various primary cancers. Furthermore, overexpression of one of these kinases transforms cells. Because of the myriad of kinases identified, we suggest a generic name: Aurora/Ipl1p-related kinase (AIRK). We denote AIRKs with a species prefix and a number, e.g. HsAIRK1.

## NOV5

Expression of gene NOV5 was assessed using the primer-probe set Ag2423, described in Table 41. Results of the RTQ-PCR runs are shown in Tables 42, 43 and 44.

**Table 41. Probe Name Ag2423**

| Primers | Sequences                                     | Length | Start Position | SEQ ID NO: |
|---------|-----------------------------------------------|--------|----------------|------------|
| Forward | 5'-aactgccactggtagacactt-3'                   | 20     | 243            | 157        |
| Probe   | TET-5'-cacactcagtgctcggttaaaattactga-3'-TAMRA | 28     | 263            | 158        |
| Reverse | 5'-tgaattcttccaccatgagaa-3'                   | 21     | 315            | 159        |

**Table 42. Panel 1.3D**

| Tissue Name | Rel. Exp.(%) Ag2423, Run 159337657 | Tissue Name | Rel. Exp.(%) Ag2423, Run 159337657 |
|-------------|------------------------------------|-------------|------------------------------------|
|-------------|------------------------------------|-------------|------------------------------------|

|                          |      |                                   |      |
|--------------------------|------|-----------------------------------|------|
| Liver adenocarcinoma     | 25.9 | Kidney (fetal)                    | 0.0  |
| Pancreas                 | 8.4  | Renal ca. 786-0                   | 11.7 |
| Pancreatic ca. CAPAN 2   | 7.1  | Renal ca. A498                    | 7.2  |
| Adrenal gland            | 65.1 | Renal ca. RXF 393                 | 9.6  |
| Thyroid                  | 4.0  | Renal ca. ACHN                    | 8.5  |
| Salivary gland           | 41.8 | Renal ca. UO-31                   | 8.4  |
| Pituitary gland          | 9.9  | Renal ca. TK-10                   | 4.5  |
| Brain (fetal)            | 75.8 | Liver                             | 13.6 |
| Brain (whole)            | 5.1  | Liver (fetal)                     | 28.1 |
| Brain (amygdala)         | 5.8  | Liver ca. (hepatoblast)<br>HepG2  | 12.9 |
| Brain (cerebellum)       | 3.8  | Lung                              | 21.0 |
| Brain (hippocampus)      | 66.4 | Lung (fetal)                      | 15.4 |
| Brain (substantia nigra) | 20.4 | Lung ca. (small cell)<br>LX-1     | 4.9  |
| Brain (thalamus)         | 7.4  | Lung ca. (small cell)<br>NCI-H69  | 4.6  |
| Cerebral Cortex          | 52.9 | Lung ca. (s.cell var.)<br>SHP-77  | 14.7 |
| Spinal cord              | 22.5 | Lung ca. (large<br>cell)NCI-H460  | 15.7 |
| glio/astro U87-MG        | 11.5 | Lung ca. (non-sm. cell)<br>A549   | 9.8  |
| glio/astro U-118-MG      | 11.1 | Lung ca. (non-s.cell)<br>NCI-H23  | 12.2 |
| astrocytoma SW1783       | 15.8 | Lung ca. (non-s.cell)<br>HOP-62   | 18.2 |
| neuro*; met SK-N-AS      | 10.2 | Lung ca. (non-s.cl)<br>NCI-H522   | 10.7 |
| astrocytoma SF-539       | 9.5  | Lung ca. (squam.) SW<br>900       | 8.0  |
| astrocytoma SNB-75       | 0.0  | Lung ca. (squam.)<br>NCI-H596     | 9.3  |
| glioma SNB-19            | 16.6 | Mammary gland                     | 13.0 |
| glioma U251              | 5.2  | Breast ca.* (pl.ef)<br>MCF-7      | 3.1  |
| glioma SF-295            | 0.0  | Breast ca.* (pl.ef)<br>MDA-MB-231 | 8.3  |
| Heart (Fetal)            | 24.1 | Breast ca.* (pl. ef)<br>T47D      | 4.0  |
| Heart                    | 33.0 | Breast ca. BT-549                 | 6.1  |
| Skeletal muscle (Fetal)  | 6.5  | Breast ca. MDA-N                  | 0.0  |
| Skeletal muscle          | 10.8 | Ovary                             | 7.2  |
| Bone marrow              | 5.7  | Ovarian ca. OVCAR-3               | 16.7 |
| Thymus                   | 0.0  | Ovarian ca. OVCAR-4               | 13.2 |
| Spleen                   | 33.0 | Ovarian ca. OVCAR-5               | 10.6 |
| Lymph node               | 13.7 | Ovarian ca. OVCAR-8               | 0.0  |
| Colorectal               | 28.5 | Ovarian ca. IGROV-1               | 9.3  |

|                                    |       |                                  |      |
|------------------------------------|-------|----------------------------------|------|
| Stomach                            | 4.8   | Ovarian ca. (ascites)<br>SK-OV-3 | 0.0  |
| Small intestine                    | 8.6   | Uterus                           | 0.0  |
| Colon ca. SW480                    | 0.0   | Placenta                         | 8.0  |
| Colon ca.* SW620<br>(SW480 met)    | 4.4   | Prostate                         | 0.0  |
| Colon ca. HT29                     | 5.3   | Prostate ca.* (bone<br>met) PC-3 | 0.0  |
| Colon ca. HCT-116                  | 19.9  | Testis                           | 0.0  |
| Colon ca. CaCo-2                   | 9.7   | Melanoma Hs688(A).T              | 0.0  |
| CC Well to Mod Diff<br>(ODO3866)   | 0.0   | Melanoma* (met)<br>Hs688(B).T    | 0.0  |
| Colon ca. HCC-2998                 | 0.0   | Melanoma UACC-62                 | 6.6  |
| Gastric ca. (liver met)<br>NCI-N87 | 0.0   | Melanoma M14                     | 5.1  |
| Bladder                            | 100.0 | Melanoma LOX IMVI                | 8.2  |
| Trachea                            | 4.4   | Melanoma* (met) SK-<br>MEL-5     | 8.7  |
| Kidney                             | 25.7  | Adipose                          | 79.6 |

Table 43. Panel 2D

| Tissue Name                                      | Rel. Exp.(%) Ag2423,<br>Run 159338041 | Tissue Name                   | Rel. Exp.(%) Ag2423,<br>Run 159338041 |
|--------------------------------------------------|---------------------------------------|-------------------------------|---------------------------------------|
| Normal Colon                                     | 34.6                                  | Kidney Margin<br>8120608      | 0.0                                   |
| CC Well to Mod Diff<br>(ODO3866)                 | 34.2                                  | Kidney Cancer 8120613         | 0.0                                   |
| CC Margin (ODO3866)                              | 38.7                                  | Kidney Margin<br>8120614      | 0.0                                   |
| CC Gr.2 rectosigmoid<br>(ODO3868)                | 9.1                                   | Kidney Cancer 9010320         | 0.0                                   |
| CC Margin (ODO3868)                              | 11.0                                  | Kidney Margin<br>9010321      | 0.0                                   |
| CC Mod Diff (ODO3920)                            | 12.9                                  | Normal Uterus                 | 8.9                                   |
| CC Margin (ODO3920)                              | 17.7                                  | Uterine Cancer 064011         | 12.2                                  |
| CC Gr.2 ascend colon<br>(ODO3921)                | 79.0                                  | Normal Thyroid                | 2.6                                   |
| CC Margin (ODO3921)                              | 17.1                                  | Thyroid Cancer                | 5.6                                   |
| CC from Partial<br>Hepatectomy (ODO4309)<br>Mets | 24.1                                  | Thyroid Cancer<br>A302152     | 7.7                                   |
| Liver Margin (ODO4309)                           | 17.8                                  | Thyroid Margin<br>A302153     | 10.9                                  |
| Colon mets to lung<br>(OD04451-01)               | 2.0                                   | Normal Breast                 | 7.2                                   |
| Lung Margin (OD04451-02)                         | 8.0                                   | Breast Cancer                 | 2.4                                   |
| Normal Prostate 6546-1                           | 2.8                                   | Breast Cancer<br>(OD04590-01) | 16.0                                  |
| Prostate Cancer (OD04410)                        | 45.4                                  | Breast Cancer Mets            | 19.5                                  |

|                                       |      |                                      |       |
|---------------------------------------|------|--------------------------------------|-------|
|                                       |      | (OD04590-03)                         |       |
| Prostate Margin (OD04410)             | 27.4 | Breast Cancer Metastasis             | 11.2  |
| Prostate Cancer (OD04720-01)          | 9.8  | Breast Cancer                        | 10.9  |
| Prostate Margin (OD04720-02)          | 29.3 | Breast Cancer                        | 3.6   |
| Normal Lung                           | 38.2 | Breast Cancer 9100266                | 12.9  |
| Lung Met to Muscle (ODO4286)          | 36.3 | Breast Margin 9100265                | 4.6   |
| Muscle Margin (ODO4286)               | 9.9  | Breast Cancer A209073                | 15.4  |
| Lung Malignant Cancer (OD03126)       | 15.7 | Breast Margin A2090734               | 6.1   |
| Lung Margin (OD03126)                 | 12.0 | Normal Liver                         | 3.8   |
| Lung Cancer (OD04404)                 | 14.4 | Liver Cancer                         | 9.1   |
| Lung Margin (OD04404)                 | 10.1 | Liver Cancer 1025                    | 3.8   |
| Lung Cancer (OD04565)                 | 7.4  | Liver Cancer 1026                    | 2.7   |
| Lung Margin (OD04565)                 | 0.0  | Liver Cancer 6004-T                  | 3.2   |
| Lung Cancer (OD04237-01)              | 43.8 | Liver Tissue 6004-N                  | 3.4   |
| Lung Margin (OD04237-02)              | 12.9 | Liver Cancer 6005-T                  | 3.4   |
| Ocular Mel Met to Liver (ODO4310)     | 3.0  | Liver Tissue 6005-N                  | 1.6   |
| Liver Margin (ODO4310)                | 4.1  | Normal Bladder                       | 36.9  |
| Melanoma Metastasis                   | 33.2 | Bladder Cancer                       | 10.0  |
| Lung Margin (OD04321)                 | 23.7 | Bladder Cancer                       | 22.4  |
| Normal Kidney                         | 12.4 | Bladder Cancer (OD04718-01)          | 100.0 |
| Kidney Ca, Nuclear grade 2 (OD04338)  | 6.8  | Bladder Normal Adjacent (OD04718-03) | 13.1  |
| Kidney Margin (OD04338)               | 6.2  | Normal Ovary                         | 3.0   |
| Kidney Ca Nuclear grade 1/2 (OD04339) | 20.7 | Ovarian Cancer                       | 18.2  |
| Kidney Margin (OD04339)               | 11.8 | Ovarian Cancer (OD04768-07)          | 47.6  |
| Kidney Ca, Clear cell type (OD04340)  | 29.9 | Ovary Margin (OD04768-08)            | 6.1   |
| Kidney Margin (OD04340)               | 11.0 | Normal Stomach                       | 6.2   |
| Kidney Ca, Nuclear grade 3 (OD04348)  | 5.8  | Gastric Cancer 9060358               | 0.0   |
| Kidney Margin (OD04348)               | 9.8  | Stomach Margin 9060359               | 42.3  |
| Kidney Cancer (OD04622-01)            | 0.0  | Gastric Cancer 9060395               | 37.4  |
| Kidney Margin (OD04622-03)            | 0.0  | Stomach Margin 9060394               | 47.0  |
| Kidney Cancer (OD04450-01)            | 7.5  | Gastric Cancer 9060397               | 76.3  |
| Kidney Margin (OD04450-               | 5.4  | Stomach Margin                       | 3.1   |

|                       |     |                       |      |
|-----------------------|-----|-----------------------|------|
| 03)                   |     | 9060396               |      |
| Kidney Cancer 8120607 | 0.0 | Gastric Cancer 064005 | 35.6 |

Table 44. Panel 4D

| Tissue Name                        | Rel. Exp.(%)<br>Ag2423, Run<br>159338325 | Tissue Name                                    | Rel. Exp.(%)<br>Ag2423, Run<br>159338325 |
|------------------------------------|------------------------------------------|------------------------------------------------|------------------------------------------|
| Secondary Th1 act                  | 2.1                                      | HUVEC IL-1beta                                 | 1.9                                      |
| Secondary Th2 act                  | 4.8                                      | HUVEC IFN gamma                                | 0.0                                      |
| Secondary Tr1 act                  | 1.4                                      | HUVEC TNF alpha + IFN<br>gamma                 | 0.0                                      |
| Secondary Th1 rest                 | 7.5                                      | HUVEC TNF alpha + IL4                          | 0.0                                      |
| Secondary Th2 rest                 | 10.2                                     | HUVEC IL-11                                    | 0.0                                      |
| Secondary Tr1 rest                 | 2.0                                      | Lung Microvascular EC none                     | 4.7                                      |
| Primary Th1 act                    | 2.3                                      | Lung Microvascular EC<br>TNFalpha + IL-1beta   | 3.5                                      |
| Primary Th2 act                    | 100.0                                    | Microvascular Dermal EC<br>none                | 8.0                                      |
| Primary Tr1 act                    | 3.4                                      | Microvascular Dermal EC<br>TNFalpha + IL-1beta | 15.3                                     |
| Primary Th1 rest                   | 0.9                                      | Bronchial epithelium<br>TNFalpha + IL1beta     | 1.3                                      |
| Primary Th2 rest                   | 3.8                                      | Small airway epithelium<br>none                | 3.6                                      |
| Primary Tr1 rest                   | 1.4                                      | Small airway epithelium<br>TNFalpha + IL-1beta | 0.0                                      |
| CD45RA CD4<br>lymphocyte act       | 1.6                                      | Coronary artery SMC rest                       | 0.0                                      |
| CD45RO CD4<br>lymphocyte act       | 0.0                                      | Coronary artery SMC<br>TNFalpha + IL-1beta     | 0.0                                      |
| CD8 lymphocyte act                 | 5.2                                      | Astrocytes rest                                | 5.1                                      |
| Secondary CD8<br>lymphocyte rest   | 0.0                                      | Astrocytes TNFalpha + IL-<br>1beta             | 0.0                                      |
| Secondary CD8<br>lymphocyte act    | 1.1                                      | KU-812 (Basophil) rest                         | 0.0                                      |
| CD4 lymphocyte none                | 2.2                                      | KU-812 (Basophil)<br>PMA/ionomycin             | 2.1                                      |
| 2ry Th1/Th2/Tr1_anti-<br>CD95 CH11 | 1.5                                      | CCD1106 (Keratinocytes)<br>none                | 1.5                                      |
| LAK cells rest                     | 7.6                                      | CCD1106 (Keratinocytes)<br>TNFalpha + IL-1beta | 2.0                                      |
| LAK cells IL-2                     | 5.8                                      | Liver cirrhosis                                | 2.1                                      |
| LAK cells IL-2+IL-12               | 1.4                                      | Lupus kidney                                   | 4.8                                      |
| LAK cells IL-2+IFN<br>gamma        | 0.0                                      | NCI-H292 none                                  | 1.6                                      |
| LAK cells IL-2+ IL-18              | 1.7                                      | NCI-H292 IL-4                                  | 6.6                                      |
| LAK cells<br>PMA/ionomycin         | 1.7                                      | NCI-H292 IL-9                                  | 0.0                                      |
| NK Cells IL-2 rest                 | 0.9                                      | NCI-H292 IL-13                                 | 0.0                                      |

|                              |      |                                       |     |
|------------------------------|------|---------------------------------------|-----|
| Two Way MLR 3 day            | 0.0  | NCI-H292 IFN gamma                    | 1.3 |
| Two Way MLR 5 day            | 1.8  | HPAEC none                            | 0.0 |
| Two Way MLR 7 day            | 8.5  | HPAEC TNF alpha + IL-1 beta           | 1.4 |
| PBMC rest                    | 1.8  | Lung fibroblast none                  | 0.9 |
| PBMC PWM                     | 1.8  | Lung fibroblast TNF alpha + IL-1 beta | 6.0 |
| PBMC PHA-L                   | 3.7  | Lung fibroblast IL-4                  | 6.3 |
| Ramos (B cell) none          | 2.0  | Lung fibroblast IL-9                  | 1.0 |
| Ramos (B cell) ionomycin     | 0.0  | Lung fibroblast IL-13                 | 0.9 |
| B lymphocytes PWM            | 0.0  | Lung fibroblast IFN gamma             | 0.8 |
| B lymphocytes CD40L and IL-4 | 0.0  | Dermal fibroblast CCD1070 rest        | 2.2 |
| EOL-1 dbcAMP                 | 4.2  | Dermal fibroblast CCD1070 TNF alpha   | 2.0 |
| EOL-1 dbcAMP PMA/ionomycin   | 0.0  | Dermal fibroblast CCD1070 IL-1 beta   | 0.0 |
| Dendritic cells none         | 2.3  | Dermal fibroblast IFN gamma           | 0.0 |
| Dendritic cells LPS          | 4.3  | Dermal fibroblast IL-4                | 0.0 |
| Dendritic cells anti-CD40    | 0.0  | IBD Colitis 2                         | 3.1 |
| Monocytes rest               | 1.7  | IBD Crohn's                           | 1.8 |
| Monocytes LPS                | 28.3 | Colon                                 | 0.0 |
| Macrophages rest             | 20.7 | Lung                                  | 0.0 |
| Macrophages LPS              | 1.1  | Thymus                                | 2.0 |
| HUVEC none                   | 2.0  | Kidney                                | 3.7 |
| HUVEC starved                | 1.5  |                                       |     |

**CNS\_neurodegeneration\_v1.0 Summary:** Ag2423 Expression is low/undetected in all samples in this panel (CT>35). (Data not shown.)

**Panel 1.3D Summary:** Ag2423 This gene is expressed exclusively in a sample derived from bladder tissue. Thus, the expression of this gene could be used to distinguish bladder tissue from other tissues in the panel.

**Panel 2D Summary:** Ag2423 The expression of this gene is highest and almost exclusive to a sample derived from bladder cancer. This result is consistent with the expression detected in Panel 1.3D. Thus, the expression of this gene could be used to distinguish bladder cancer tissue from other tissues in the panel. Moreover, the therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be of benefit in the treatment of bladder cancer.

**Panel 4D Summary:** Ag2423 The expression of this gene is highest and almost exclusive to primary activated Th2 cells (CT 32.6). Very low expression of this transcript is found in activated LPS and macrophages (CT 34.9). This transcript encodes for a 26s proteasome like protein which is an essential component of the cellular protein degradation machinery. Some studies (reference 1) indicate a potential role for proteasomes in the regulation of signal transduction in T and B lymphocytes. This novel 26S proteasome may be involved in a more specific Th2 signalling pathway. Therefore, this gene product may be useful as a potential therapeutic target for attenuation of hyperactive Th2 response such as observed in allergic diseases (rhinitis, atopic skin diseases, asthma).

Reference:

Biochim Biophys Acta 1999 Jan 6;1453(1):92-104 Proteasome participates in the alteration of signal transduction in T and B lymphocytes following trauma-hemorrhage. Samy TS, Schwacha MG, Chung CS, Cioffi WG, Bland KI, Chaudry IH.

Department of Surgery, Brown University School of Medicine, Providence, RI, USA.

Proteasomes are essential components of the cellular protein degradation machinery. They are nonlysosomal and their participation is critical for (1) the removal of short lived proteins involved in metabolic regulation and cell proliferation, (2) the control of the activities of regulators involved in gene transcription, such as nuclear factor-kappa B (NF-kappa B) and signal transducer and activator of transcription (STAT1), and (3) processing of antigenic peptides for MHC class I presentation. Trauma-hemorrhage induces profound immunosuppression which is characterized by reduced splenocyte proliferation, interleukin (IL)-2 and interferon (IFN)-gamma productive capacity, increased activation of transcription factors NF-kappa B and STAT1 in splenic T lymphocytes, reduced macrophage antigen presentation capacity and inordinate release of proinflammatory cytokines, such as IL-6 and tumor necrosis factor-alpha. Furthermore, it appears that the activity of several regulatory proteins involved in immune function is altered by trauma-hemorrhage. Since proteasomes are involved in regulation and removal of regulatory proteins, we hypothesized that trauma-hemorrhage alters proteasomal activity in splenic lymphocytes. The data showed that activities of 26s proteasome from CD3+CD4+ and CD3+CD8+ splenic T lymphocytes were enhanced following trauma-hemorrhage which was associated with increased expression of NF-kappa B and STAT1. On the other hand, trauma-hemorrhage attenuated the activity of 26s proteasome from splenic B lymphocytes which was restored upon IFN-gamma stimulation and correlated with increased expression of NF-kappa B. These studies indicate a potential role for proteasomes in the regulation of signal transduction in splenic T and B lymphocytes following



trauma-hemorrhage, and also suggest them as potential therapeutic targets for attenuation of immune suppression associated with this form of injury.

## NOV6

Expression of gene NOV6 was assessed using the primer-probe sets Ag1508, Ag1586, Ag2011 and Ag2284, described in Tables 45, 46, 47 and 48. Results of the RTQ-PCR runs are shown in Tables 49, 50, 51, 52, 53 and 54.

**Table 45. Probe Name Ag1508**

| Primers | Sequences                               | Length | Start Position | SEQ ID NO: |
|---------|-----------------------------------------|--------|----------------|------------|
| Forward | 5'-atttggctatcccttcagggtt-3'            | 21     | 238            | 160        |
| Probe   | TET-5'-cggatccaatagagatgccctct-3'-TAMRA | 25     | 263            | 161        |
| Reverse | 5'-gtcttggagctggactcttcat-3'            | 22     | 291            | 162        |

**Table 46. Probe Name Ag1586**

| Primers | Sequences                                 | Length | Start Position | SEQ ID NO: |
|---------|-------------------------------------------|--------|----------------|------------|
| Forward | 5'-accaggatgagtttgtgtcatc-3'              | 22     | 1583           | 163        |
| Probe   | TET-5'-ctcaagatcccttcggacacgctgt-3'-TAMRA | 25     | 1609           | 164        |
| Reverse | 5'-tgcggaagctgtacacatagta-3'              | 22     | 1657           | 165        |

**Table 47. Probe Name Ag2011**

| Primers | Sequences                                 | Length | Start Position | SEQ ID NO: |
|---------|-------------------------------------------|--------|----------------|------------|
| Forward | 5'-accaggatgagtttgtgtcatc-3'              | 22     | 1583           | 166        |
| Probe   | TET-5'-ctcaagatcccttcggacacgctgt-3'-TAMRA | 25     | 1609           | 167        |
| Reverse | 5'-tgcggaagctgtacacatagta-3'              | 22     | 1657           | 168        |

**Table 48. Probe Name Ag2284**

| Primers | Sequences                                  | Length | Start Position | SEQ ID NO" |
|---------|--------------------------------------------|--------|----------------|------------|
| Forward | 5'-tagttatctacctgcgcttcca-3'               | 22     | 399            | 169        |
| Probe   | TET-5'-tctacacagagaacaaacgcttcccg-3'-TAMRA | 26     | 426            | 170        |
| Reverse | 5'-gaaggtgaaggagacagtcaca-3'               | 22     | 466            | 171        |

**Table 49. Panel 1.2**

| Tissue Name       | Rel. Exp.(%) Ag1508, Run 141937122 | Tissue Name       | Rel. Exp.(%) Ag1508, Run 141937122 |
|-------------------|------------------------------------|-------------------|------------------------------------|
| Endothelial cells | 0.0                                | Renal ca. 786-0   | 0.0                                |
| Heart (Fetal)     | 0.9                                | Renal ca. A498    | 0.0                                |
| Pancreas          | 0.1                                | Renal ca. RXF 393 | 0.0                                |

|                        |       |                                   |     |
|------------------------|-------|-----------------------------------|-----|
| Pancreatic ca. CAPAN 2 | 0.0   | Renal ca. ACHN                    | 0.0 |
| Adrenal Gland          | 2.7   | Renal ca. UO-31                   | 0.0 |
| Thyroid                | 0.1   | Renal ca. TK-10                   | 0.0 |
| Salivary gland         | 0.9   | Liver                             | 0.3 |
| Pituitary gland        | 0.0   | Liver (fetal)                     | 0.1 |
| Brain (fetal)          | 0.0   | Liver ca. (hepatoblast)<br>HepG2  | 0.0 |
| Brain (whole)          | 0.0   | Lung                              | 0.0 |
| Brain (amygdala)       | 0.0   | Lung (fetal)                      | 0.0 |
| Brain (cerebellum)     | 0.1   | Lung ca. (small cell)<br>LX-1     | 0.0 |
| Brain (hippocampus)    | 0.1   | Lung ca. (small cell)<br>NCI-H69  | 0.0 |
| Brain (thalamus)       | 0.0   | Lung ca. (s.cell var.)<br>SHP-77  | 0.0 |
| Cerebral Cortex        | 0.3   | Lung ca. (large<br>cell)NCI-H460  | 0.0 |
| Spinal cord            | 0.0   | Lung ca. (non-sm. cell)<br>A549   | 0.0 |
| glio/astro U87-MG      | 0.0   | Lung ca. (non-s.cell)<br>NCI-H23  | 0.0 |
| glio/astro U-118-MG    | 0.1   | Lung ca. (non-s.cell)<br>HOP-62   | 0.0 |
| astrocytoma SW1783     | 0.0   | Lung ca. (non-s.cl)<br>NCI-H522   | 9.4 |
| neuro*; met SK-N-AS    | 0.0   | Lung ca. (squam.) SW<br>900       | 0.2 |
| astrocytoma SF-539     | 0.0   | Lung ca. (squam.)<br>NCI-H596     | 0.0 |
| astrocytoma SNB-75     | 0.0   | Mammary gland                     | 0.0 |
| glioma SNB-19          | 0.0   | Breast ca.* (pl.ef)<br>MCF-7      | 0.0 |
| glioma U251            | 0.0   | Breast ca.* (pl.ef)<br>MDA-MB-231 | 0.0 |
| glioma SF-295          | 0.0   | Breast ca.* (pl. ef)<br>T47D      | 0.0 |
| Heart                  | 10.7  | Breast ca. BT-549                 | 0.0 |
| Skeletal Muscle        | 100.0 | Breast ca. MDA-N                  | 0.0 |
| Bone marrow            | 0.1   | Ovary                             | 0.5 |
| Thymus                 | 0.0   | Ovarian ca. OVCAR-3               | 0.0 |
| Spleen                 | 0.0   | Ovarian ca. OVCAR-4               | 0.0 |
| Lymph node             | 0.0   | Ovarian ca. OVCAR-5               | 0.0 |
| Colorectal             | 0.0   | Ovarian ca. OVCAR-8               | 0.0 |
| Stomach                | 0.1   | Ovarian ca. IGROV-1               | 0.0 |
| Small intestine        | 0.2   | Ovarian ca. (ascites)<br>SK-OV-3  | 0.0 |
| Colon ca. SW480        | 0.0   | Uterus                            | 0.2 |
| Colon ca.* SW620       | 0.0   | Placenta                          | 0.0 |

|                                 |     |                               |     |
|---------------------------------|-----|-------------------------------|-----|
| (SW480 met)                     |     |                               |     |
| Colon ca. HT29                  | 0.0 | Prostate                      | 0.4 |
| Colon ca. HCT-116               | 0.1 | Prostate ca.* (bone met) PC-3 | 0.0 |
| Colon ca. CaCo-2                | 0.0 | Testis                        | 0.2 |
| CC Well to Mod Diff (ODO3866)   | 0.0 | Melanoma Hs688(A).T           | 0.0 |
| Colon ca. HCC-2998              | 0.0 | Melanoma* (met) Hs688(B).T    | 0.0 |
| Gastric ca. (liver met) NCI-N87 | 0.0 | Melanoma UACC-62              | 0.1 |
| Bladder                         | 0.2 | Melanoma M14                  | 0.0 |
| Trachea                         | 0.0 | Melanoma LOX IMVI             | 0.0 |
| Kidney                          | 8.9 | Melanoma* (met) SK-MEL-5      | 0.0 |
| Kidney (fetal)                  | 0.6 |                               |     |

Table 50. Panel 1.3D

| Tissue Name              | Rel. Exp.(%)<br>Ag1586,<br>Run<br>146473155 | Rel. Exp.(%)<br>Ag2011,<br>Run<br>147816085 | Rel. Exp.(%)<br>Ag2284,<br>Run<br>167985231 | Tissue Name                   | Rel. Exp.(%)<br>Ag1586,<br>Run<br>146473155 | Rel. Exp.(%)<br>Ag2011,<br>Run<br>147816085 | Rel. Exp.(%)<br>Ag2284,<br>Run<br>167985231 |
|--------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|-------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| Liver adenocarcinoma     | 29.9                                        | 37.6                                        | 0.2                                         | Kidney (fetal)                | 3.8                                         | 3.7                                         | 1.6                                         |
| Pancreas                 | 1.7                                         | 0.7                                         | 0.3                                         | Renal ca. 786-0               | 6.1                                         | 11.7                                        | 0.0                                         |
| Pancreatic ca. CAPAN 2   | 6.3                                         | 9.6                                         | 0.0                                         | Renal ca. A498                | 25.0                                        | 25.9                                        | 0.0                                         |
| Adrenal gland            | 2.6                                         | 2.5                                         | 0.5                                         | Renal ca. RXF 393             | 4.5                                         | 5.0                                         | 0.0                                         |
| Thyroid                  | 2.5                                         | 1.8                                         | 1.2                                         | Renal ca. ACHN                | 8.8                                         | 11.3                                        | 0.0                                         |
| Salivary gland           | 1.9                                         | 2.2                                         | 0.4                                         | Renal ca. UO-31               | 15.0                                        | 15.0                                        | 0.0                                         |
| Pituitary gland          | 0.9                                         | 1.5                                         | 0.1                                         | Renal ca. TK-10               | 4.4                                         | 4.6                                         | 0.0                                         |
| Brain (fetal)            | 12.2                                        | 13.1                                        | 0.0                                         | Liver                         | 0.2                                         | 0.1                                         | 0.4                                         |
| Brain (whole)            | 9.7                                         | 10.7                                        | 0.2                                         | Liver (fetal)                 | 0.7                                         | 0.8                                         | 0.1                                         |
| Brain (amygdala)         | 9.5                                         | 9.9                                         | 0.2                                         | Liver ca. (hepatoblast) HepG2 | 16.8                                        | 12.8                                        | 0.1                                         |
| Brain (cerebellum)       | 3.3                                         | 2.3                                         | 0.1                                         | Lung                          | 5.0                                         | 5.1                                         | 0.0                                         |
| Brain (hippocampus)      | 24.7                                        | 21.0                                        | 0.1                                         | Lung (fetal)                  | 7.4                                         | 8.1                                         | 0.1                                         |
| Brain (substantia nigra) | 0.9                                         | 1.3                                         | 0.1                                         | Lung ca. (small cell) LX-1    | 16.8                                        | 12.1                                        | 0.0                                         |

|                         |      |      |       |                                |      |      |     |
|-------------------------|------|------|-------|--------------------------------|------|------|-----|
| Brain (thalamus)        | 4.7  | 3.7  | 0.1   | Lung ca. (small cell) NCI-H69  | 18.4 | 23.7 | 0.0 |
| Cerebral Cortex         | 75.8 | 71.2 | 0.2   | Lung ca. (s.cell var.) SHP-77  | 8.5  | 7.2  | 0.0 |
| Spinal cord             | 2.0  | 2.4  | 0.1   | Lung ca. (large cell) NCI-H460 | 10.7 | 10.1 | 0.0 |
| glio/astro U87-MG       | 15.3 | 17.9 | 0.0   | Lung ca. (non-sm. cell) A549   | 3.2  | 4.1  | 0.0 |
| glio/astro U-118-MG     | 38.2 | 41.2 | 0.2   | Lung ca. (non-s.cell) NCI-H23  | 23.2 | 24.7 | 0.5 |
| astrocytoma SW1783      | 8.3  | 10.4 | 0.1   | Lung ca. (non-s.cell) HOP-62   | 18.9 | 15.7 | 0.0 |
| neuro*; met SK-N-AS     | 23.5 | 24.3 | 0.0   | Lung ca. (non-s.cl) NCI-H522   | 5.6  | 7.5  | 8.1 |
| astrocytoma SF-539      | 19.6 | 38.4 | 0.0   | Lung ca. (squam.) SW 900       | 13.0 | 13.1 | 0.2 |
| astrocytoma SNB-75      | 44.4 | 45.1 | 0.1   | Lung ca. (squam.) NCI-H596     | 6.5  | 5.7  | 0.0 |
| glioma SNB-19           | 26.2 | 12.2 | 0.0   | Mammary gland                  | 11.5 | 9.3  | 0.2 |
| glioma U251             | 16.4 | 16.2 | 0.1   | Breast ca.* (pl.ef) MCF-7      | 14.1 | 14.4 | 0.0 |
| glioma SF-295           | 26.4 | 36.9 | 0.0   | Breast ca.* (pl.ef) MDA-MB-231 | 82.9 | 87.1 | 0.0 |
| Heart (Fetal)           | 80.7 | 95.3 | 1.8   | Breast ca.* (pl. ef) T47D      | 6.1  | 4.6  | 0.1 |
| Heart                   | 2.8  | 1.9  | 2.3   | Breast ca. BT-549              | 13.6 | 11.2 | 0.2 |
| Skeletal muscle (Fetal) | 85.3 | 87.7 | 100.0 | Breast ca. MDA-N               | 28.1 | 31.6 | 0.0 |
| Skeletal muscle         | 2.1  | 2.4  | 88.3  | Ovary                          | 20.9 | 19.5 | 0.8 |
| Bone marrow             | 0.6  | 0.3  | 0.2   | Ovarian ca. OVCAR-3            | 33.0 | 40.1 | 0.0 |
| Thymus                  | 2.6  | 2.3  | 0.0   | Ovarian ca. OVCAR-4            | 5.5  | 5.4  | 0.0 |
| Spleen                  | 2.9  | 2.6  | 0.0   | Ovarian ca. OVCAR-5            | 10.9 | 13.1 | 0.1 |

|                                 |      |      |     |                               |       |       |     |
|---------------------------------|------|------|-----|-------------------------------|-------|-------|-----|
| Lymph node                      | 5.1  | 5.2  | 0.1 | Ovarian ca. OVCAR-8           | 17.4  | 18.3  | 0.1 |
| Colorectal                      | 5.2  | 3.9  | 0.0 | Ovarian ca. IGROV-1           | 4.5   | 5.3   | 0.0 |
| Stomach                         | 3.7  | 5.6  | 0.2 | Ovarian ca. (ascites) SK-OV-3 | 25.7  | 22.4  | 0.1 |
| Small intestine                 | 1.6  | 1.3  | 0.2 | Uterus                        | 2.7   | 2.4   | 1.0 |
| Colon ca. SW480                 | 45.4 | 55.5 | 0.1 | Placenta                      | 6.7   | 10.2  | 0.2 |
| Colon ca.* SW620 (SW480 met)    | 11.3 | 11.1 | 0.0 | Prostate                      | 0.4   | 1.4   | 0.2 |
| Colon ca. HT29                  | 13.3 | 13.3 | 0.0 | Prostate ca.* (bone met) PC-3 | 8.4   | 11.3  | 0.0 |
| Colon ca. HCT-116               | 10.5 | 10.5 | 0.2 | Testis                        | 8.1   | 8.5   | 1.1 |
| Colon ca. CaCo-2                | 24.0 | 23.0 | 0.1 | Melanoma Hs688(A).T           | 59.0  | 86.5  | 0.0 |
| CC Well to Mod Diff (ODO3866)   | 19.1 | 16.6 | 0.1 | Melanoma* (met) Hs688(B).T    | 100.0 | 100.0 | 0.0 |
| Colon ca. HCC-2998              | 25.7 | 20.3 | 0.0 | Melanoma UACC-62              | 17.6  | 19.5  | 0.1 |
| Gastric ca. (liver met) NCI-N87 | 59.9 | 62.9 | 0.1 | Melanoma M14                  | 16.3  | 21.9  | 0.0 |
| Bladder                         | 1.8  | 4.6  | 0.2 | Melanoma LOX IMVI             | 3.6   | 5.8   | 0.0 |
| Trachea                         | 6.9  | 5.6  | 0.1 | Melanoma* (met) SK-MEL-5      | 12.9  | 22.1  | 0.0 |
| Kidney                          | 0.8  | 0.7  | 2.8 | Adipose                       | 5.6   | 4.5   | 0.7 |

Table 51. Panel 2.2

| Tissue Name               | Rel. Exp.(%) Ag2011, Run 174154748 | Tissue Name                              | Rel. Exp.(%) Ag2011, Run 174154748 |
|---------------------------|------------------------------------|------------------------------------------|------------------------------------|
| Normal Colon              | 24.7                               | Kidney Margin (OD04348)                  | 68.3                               |
| Colon cancer (OD06064)    | 48.6                               | Kidney malignant cancer (OD06204B)       | 25.0                               |
| Colon Margin (OD06064)    | 4.9                                | Kidney normal adjacent tissue (OD06204E) | 7.4                                |
| Colon cancer (OD06159)    | 9.3                                | Kidney Cancer (OD04450-01)               | 34.4                               |
| Colon Margin (OD06159)    | 19.5                               | Kidney Margin (OD04450-03)               | 18.4                               |
| Colon cancer (OD06297-04) | 11.7                               | Kidney Cancer 8120613                    | 9.7                                |

|                                             |       |                                         |      |
|---------------------------------------------|-------|-----------------------------------------|------|
| Colon Margin (OD06297-015)                  | 12.5  | Kidney Margin 8120614                   | 18.8 |
| CC Gr.2 ascend colon (ODO3921)              | 17.3  | Kidney Cancer 9010320                   | 16.2 |
| CC Margin (ODO3921)                         | 14.2  | Kidney Margin 9010321                   | 13.8 |
| Colon cancer metastasis (OD06104)           | 8.6   | Kidney Cancer 8120607                   | 37.1 |
| Lung Margin (OD06104)                       | 8.3   | Kidney Margin 8120608                   | 7.0  |
| Colon mets to lung (OD04451-01)             | 23.0  | Normal Uterus                           | 21.9 |
| Lung Margin (OD04451-02)                    | 32.8  | Uterine Cancer 064011                   | 13.7 |
| Normal Prostate                             | 4.8   | Normal Thyroid                          | 2.4  |
| Prostate Cancer (OD04410)                   | 4.9   | Thyroid Cancer                          | 8.1  |
| Prostate Margin (OD04410)                   | 8.8   | Thyroid Cancer A302152                  | 35.4 |
| Normal Ovary                                | 32.3  | Thyroid Margin A302153                  | 8.7  |
| Ovarian cancer (OD06283-03)                 | 32.1  | Normal Breast                           | 29.7 |
| Ovarian Margin (OD06283-07)                 | 13.8  | Breast Cancer                           | 11.9 |
| Ovarian Cancer                              | 19.9  | Breast Cancer                           | 47.6 |
| Ovarian cancer (OD06145)                    | 9.2   | Breast Cancer (OD04590-01)              | 25.5 |
| Ovarian Margin (OD06145)                    | 8.6   | Breast Cancer Mets (OD04590-03)         | 38.4 |
| Ovarian cancer (OD06455-03)                 | 13.0  | Breast Cancer Metastasis                | 30.1 |
| Ovarian Margin (OD06455-07)                 | 2.1   | Breast Cancer                           | 41.5 |
| Normal Lung                                 | 27.2  | Breast Cancer 9100266                   | 9.2  |
| Invasive poor diff. lung adeno (ODO4945-01) | 28.5  | Breast Margin 9100265                   | 18.2 |
| Lung Margin (ODO4945-03)                    | 15.0  | Breast Cancer A209073                   | 14.9 |
| Lung Malignant Cancer (OD03126)             | 30.4  | Breast Margin A2090734                  | 37.6 |
| Lung Margin (OD03126)                       | 15.9  | Breast cancer (OD06083)                 | 55.9 |
| Lung Cancer (OD05014A)                      | 39.5  | Breast cancer node metastasis (OD06083) | 48.6 |
| Lung Margin (OD05014B)                      | 22.1  | Normal Liver                            | 10.4 |
| Lung cancer (OD06081)                       | 23.7  | Liver Cancer 1026                       | 9.1  |
| Lung Margin (OD06081)                       | 16.8  | Liver Cancer 1025                       | 20.7 |
| Lung Cancer (OD04237-01)                    | 9.0   | Liver Cancer 6004-T                     | 12.2 |
| Lung Margin (OD04237-02)                    | 41.5  | Liver Tissue 6004-N                     | 8.0  |
| Ocular Mel Met to Liver                     | 100.0 | Liver Cancer 6005-T                     | 36.6 |

|                                       |      |                        |      |
|---------------------------------------|------|------------------------|------|
| (ODO4310)                             |      |                        |      |
| Liver Margin (ODO4310)                | 4.2  | Liver Tissue 6005-N    | 25.0 |
| Melanoma Metastasis                   | 47.0 | Liver Cancer           | 4.5  |
| Lung Margin (ODO4321)                 | 28.1 | Normal Bladder         | 18.7 |
| Normal Kidney                         | 12.3 | Bladder Cancer         | 17.2 |
| Kidney Ca, Nuclear grade 2 (ODO4338)  | 18.3 | Bladder Cancer         | 72.7 |
| Kidney Margin (ODO4338)               | 18.0 | Normal Stomach         | 33.4 |
| Kidney Ca Nuclear grade 1/2 (ODO4339) | 83.5 | Gastric Cancer 9060397 | 9.6  |
| Kidney Margin (ODO4339)               | 10.4 | Stomach Margin 9060396 | 10.4 |
| Kidney Ca, Clear cell type (ODO4340)  | 22.2 | Gastric Cancer 9060395 | 7.6  |
| Kidney Margin (ODO4340)               | 12.7 | Stomach Margin 9060394 | 19.6 |
| Kidney Ca, Nuclear grade 3 (ODO4348)  | 15.7 | Gastric Cancer 064005  | 17.4 |

Table 52. Panel 2D

| Tissue Name                                | Rel. Exp.(%)<br>Ag1508, Run<br>144982575 | Rel. Exp.(%)<br>Ag1586, Run<br>162624476 | Tissue Name            | Rel. Exp.(%)<br>Ag1508, Run<br>144982575 | Rel. Exp.(%)<br>Ag1586, Run<br>162624476 |
|--------------------------------------------|------------------------------------------|------------------------------------------|------------------------|------------------------------------------|------------------------------------------|
| Normal Colon                               | 2.2                                      | 34.9                                     | Kidney Margin 8120608  | 11.3                                     | 14.2                                     |
| CC Well to Mod Diff (ODO3866)              | 0.1                                      | 28.3                                     | Kidney Cancer 8120613  | 3.6                                      | 30.4                                     |
| CC Margin (ODO3866)                        | 1.4                                      | 9.2                                      | Kidney Margin 8120614  | 11.0                                     | 17.7                                     |
| CC Gr.2 rectosigmoid (ODO3868)             | 0.1                                      | 25.9                                     | Kidney Cancer 9010320  | 0.7                                      | 57.0                                     |
| CC Margin (ODO3868)                        | 0.6                                      | 4.7                                      | Kidney Margin 9010321  | 12.0                                     | 40.9                                     |
| CC Mod Diff (ODO3920)                      | 0.1                                      | 55.5                                     | Normal Uterus          | 2.8                                      | 10.4                                     |
| CC Margin (ODO3920)                        | 1.1                                      | 14.2                                     | Uterine Cancer 064011  | 0.6                                      | 28.9                                     |
| CC Gr.2 ascend colon (ODO3921)             | 0.1                                      | 62.9                                     | Normal Thyroid         | 15.1                                     | 8.4                                      |
| CC Margin (ODO3921)                        | 0.6                                      | 12.1                                     | Thyroid Cancer         | 7.1                                      | 16.7                                     |
| CC from Partial Hepatectomy (ODO4309) Mets | 0.3                                      | 41.5                                     | Thyroid Cancer A302152 | 0.9                                      | 24.7                                     |
| Liver Margin (ODO4309)                     | 2.4                                      | 13.6                                     | Thyroid Margin A302153 | 3.1                                      | 17.7                                     |
| Colon mets to lung (ODO4451-01)            | 0.2                                      | 18.0                                     | Normal Breast          | 0.3                                      | 60.3                                     |

|                                      |       |       |                                      |     |      |
|--------------------------------------|-------|-------|--------------------------------------|-----|------|
| Lung Margin (OD04451-02)             | 0.4   | 25.5  | Breast Cancer                        | 0.0 | 24.1 |
| Normal Prostate 6546-1               | 3.3   | 17.0  | Breast Cancer (OD04590-01)           | 0.2 | 47.0 |
| Prostate Cancer (OD04410)            | 3.4   | 33.7  | Breast Cancer Mets (OD04590-03)      | 0.7 | 72.7 |
| Prostate Margin (OD04410)            | 0.5   | 28.9  | Breast Cancer Metastasis             | 0.0 | 37.4 |
| Prostate Cancer (OD04720-01)         | 0.3   | 33.7  | Breast Cancer                        | 0.2 | 36.9 |
| Prostate Margin (OD04720-02)         | 2.6   | 45.7  | Breast Cancer                        | 0.1 | 65.1 |
| Normal Lung                          | 0.7   | 80.7  | Breast Cancer 9100266                | 0.4 | 39.8 |
| Lung Met to Muscle (ODO4286)         | 0.3   | 100.0 | Breast Margin 9100265                | 0.3 | 31.2 |
| Muscle Margin (ODO4286)              | 100.0 | 21.5  | Breast Cancer A209073                | 0.2 | 49.0 |
| Lung Malignant Cancer (OD03126)      | 0.3   | 57.8  | Breast Margin A2090734               | 0.0 | 44.8 |
| Lung Margin (OD03126)                | 0.4   | 61.6  | Normal Liver                         | 1.6 | 4.5  |
| Lung Cancer (OD04404)                | 0.1   | 70.2  | Liver Cancer                         | 0.9 | 2.6  |
| Lung Margin (OD04404)                | 0.3   | 34.2  | Liver Cancer 1025                    | 1.1 | 4.7  |
| Lung Cancer (OD04565)                | 0.0   | 87.7  | Liver Cancer 1026                    | 1.0 | 18.3 |
| Lung Margin (OD04565)                | 0.8   | 23.8  | Liver Cancer 6004-T                  | 2.3 | 7.6  |
| Lung Cancer (OD04237-01)             | 0.2   | 41.5  | Liver Tissue 6004-N                  | 0.3 | 12.0 |
| Lung Margin (OD04237-02)             | 0.5   | 34.2  | Liver Cancer 6005-T                  | 0.7 | 12.1 |
| Ocular Mel Met to Liver (ODO4310)    | 1.3   | 97.3  | Liver Tissue 6005-N                  | 1.6 | 5.7  |
| Liver Margin (ODO4310)               | 3.2   | 5.0   | Normal Bladder                       | 0.9 | 38.2 |
| Melanoma Metastasis                  | 0.0   | 87.7  | Bladder Cancer                       | 0.0 | 21.3 |
| Lung Margin (OD04321)                | 0.6   | 56.3  | Bladder Cancer                       | 0.1 | 46.0 |
| Normal Kidney                        | 18.8  | 30.1  | Bladder Cancer (OD04718-01)          | 0.2 | 96.6 |
| Kidney Ca, Nuclear grade 2 (OD04338) | 7.5   | 46.7  | Bladder Normal Adjacent (OD04718-03) | 2.9 | 29.5 |



|                                       |      |      |                             |     |      |
|---------------------------------------|------|------|-----------------------------|-----|------|
| Kidney Margin (OD04338)               | 6.0  | 14.8 | Normal Ovary                | 1.1 | 21.5 |
| Kidney Ca Nuclear grade 1/2 (OD04339) | 11.3 | 52.1 | Ovarian Cancer              | 0.3 | 73.7 |
| Kidney Margin (OD04339)               | 14.2 | 20.3 | Ovarian Cancer (OD04768-07) | 0.0 | 48.3 |
| Kidney Ca, Clear cell type (OD04340)  | 2.5  | 49.0 | Ovary Margin (OD04768-08)   | 0.2 | 18.8 |
| Kidney Margin (OD04340)               | 11.4 | 23.2 | Normal Stomach              | 0.9 | 13.9 |
| Kidney Ca, Nuclear grade 3 (OD04348)  | 0.9  | 42.6 | Gastric Cancer 9060358      | 0.3 | 6.7  |
| Kidney Margin (OD04348)               | 9.3  | 28.9 | Stomach Margin 9060359      | 0.3 | 13.2 |
| Kidney Cancer (OD04622-01)            | 0.4  | 50.7 | Gastric Cancer 9060395      | 1.3 | 28.3 |
| Kidney Margin (OD04622-03)            | 1.7  | 8.6  | Stomach Margin 9060394      | 0.4 | 18.0 |
| Kidney Cancer (OD04450-01)            | 6.2  | 21.8 | Gastric Cancer 9060397      | 0.4 | 45.4 |
| Kidney Margin (OD04450-03)            | 6.1  | 18.2 | Stomach Margin 9060396      | 0.0 | 10.4 |
| Kidney Cancer 8120607                 | 0.9  | 25.0 | Gastric Cancer 064005       | 0.5 | 48.3 |

Table 53. Panel 4.1D

| Tissue Name        | Rel. Exp.(%)<br>Ag2284, Run<br>170069125 | Tissue Name                                 | Rel. Exp.(%)<br>Ag2284, Run<br>170069125 |
|--------------------|------------------------------------------|---------------------------------------------|------------------------------------------|
| Secondary Th1 act  | 0.0                                      | HUVEC IL-1beta                              | 0.0                                      |
| Secondary Th2 act  | 1.0                                      | HUVEC IFN gamma                             | 0.0                                      |
| Secondary Tr1 act  | 0.0                                      | HUVEC TNF alpha + IFN gamma                 | 0.0                                      |
| Secondary Th1 rest | 0.7                                      | HUVEC TNF alpha + IL4                       | 0.0                                      |
| Secondary Th2 rest | 0.5                                      | HUVEC IL-11                                 | 0.0                                      |
| Secondary Tr1 rest | 0.0                                      | Lung Microvascular EC none                  | 0.0                                      |
| Primary Th1 act    | 0.0                                      | Lung Microvascular EC TNFalpha + IL-1beta   | 0.0                                      |
| Primary Th2 act    | 0.7                                      | Microvascular Dermal EC none                | 0.0                                      |
| Primary Tr1 act    | 0.0                                      | Microvascular Dermal EC TNFalpha + IL-1beta | 0.0                                      |
| Primary Th1 rest   | 0.0                                      | Bronchial epithelium TNFalpha + IL1beta     | 1.0                                      |

|                                |     |                                             |      |
|--------------------------------|-----|---------------------------------------------|------|
| Primary Th2 rest               | 0.0 | Small airway epithelium none                | 0.0  |
| Primary Tr1 rest               | 0.0 | Small airway epithelium TNFalpha + IL-1beta | 0.0  |
| CD45RA CD4 lymphocyte act      | 7.5 | Coronary artery SMC rest                    | 0.0  |
| CD45RO CD4 lymphocyte act      | 0.0 | Coronary artery SMC TNFalpha + IL-1beta     | 0.0  |
| CD8 lymphocyte act             | 0.0 | Astrocytes rest                             | 1.9  |
| Secondary CD8 lymphocyte rest  | 0.0 | Astrocytes TNFalpha + IL-1beta              | 3.2  |
| Secondary CD8 lymphocyte act   | 0.0 | KU-812 (Basophil) rest                      | 0.0  |
| CD4 lymphocyte none            | 0.0 | KU-812 (Basophil) PMA/ionomycin             | 0.9  |
| 2ry Th1/Th2/Tr1_anti-CD95 CH11 | 1.2 | CCD1106 (Keratinocytes) none                | 0.0  |
| LAK cells rest                 | 0.8 | CCD1106 (Keratinocytes) TNFalpha + IL-1beta | 0.0  |
| LAK cells IL-2                 | 0.0 | Liver cirrhosis                             | 2.2  |
| LAK cells IL-2+IL-12           | 0.4 | NCI-H292 none                               | 0.8  |
| LAK cells IL-2+IFN gamma       | 0.0 | NCI-H292 IL-4                               | 0.0  |
| LAK cells IL-2+ IL-18          | 0.0 | NCI-H292 IL-9                               | 0.0  |
| LAK cells PMA/ionomycin        | 1.5 | NCI-H292 IL-13                              | 0.0  |
| NK Cells IL-2 rest             | 1.3 | NCI-H292 IFN gamma                          | 0.0  |
| Two Way MLR 3 day              | 1.3 | HPAEC none                                  | 0.0  |
| Two Way MLR 5 day              | 1.8 | HPAEC TNF alpha + IL-1 beta                 | 0.0  |
| Two Way MLR 7 day              | 0.0 | Lung fibroblast none                        | 27.9 |
| PBMC rest                      | 0.0 | Lung fibroblast TNF alpha + IL-1 beta       | 4.7  |
| PBMC PWM                       | 0.9 | Lung fibroblast IL-4                        | 19.3 |
| PBMC PHA-L                     | 0.0 | Lung fibroblast IL-9                        | 32.3 |
| Ramos (B cell) none            | 0.0 | Lung fibroblast IL-13                       | 11.4 |
| Ramos (B cell) ionomycin       | 0.0 | Lung fibroblast IFN gamma                   | 9.9  |
| B lymphocytes PWM              | 0.8 | Dermal fibroblast CCD1070 rest              | 43.2 |
| B lymphocytes CD40L and IL-4   | 0.0 | Dermal fibroblast CCD1070 TNF alpha         | 31.0 |
| EOL-1 dbcAMP                   | 0.0 | Dermal fibroblast CCD1070 IL-1 beta         | 7.4  |
| EOL-1 dbcAMP PMA/ionomycin     | 0.0 | Dermal fibroblast IFN gamma                 | 5.8  |
| Dendritic cells none           | 0.0 | Dermal fibroblast IL-4                      | 38.4 |
| Dendritic cells LPS            | 0.5 | Dermal Fibroblasts rest                     | 24.7 |

|                           |     |                      |       |
|---------------------------|-----|----------------------|-------|
| Dendritic cells anti-CD40 | 0.9 | Neutrophils TNFa+LPS | 0.0   |
| Monocytes rest            | 0.0 | Neutrophils rest     | 0.0   |
| Monocytes LPS             | 2.4 | Colon                | 1.0   |
| Macrophages rest          | 8.9 | Lung                 | 7.3   |
| Macrophages LPS           | 0.0 | Thymus               | 3.1   |
| HUVEC none                | 0.0 | Kidney               | 100.0 |
| HUVEC starved             | 0.0 |                      |       |

Table 54. Panel 4D

| Tissue Name                    | Rel. Exp.(%)<br>Ag2011, Run<br>160997385 | Tissue Name                                 | Rel. Exp.(%)<br>Ag2011, Run<br>160997385 |
|--------------------------------|------------------------------------------|---------------------------------------------|------------------------------------------|
| Secondary Th1 act              | 4.7                                      | HUVEC IL-1beta                              | 2.0                                      |
| Secondary Th2 act              | 6.4                                      | HUVEC IFN gamma                             | 4.0                                      |
| Secondary Tr1 act              | 8.6                                      | HUVEC TNF alpha + IFN gamma                 | 5.0                                      |
| Secondary Th1 rest             | 0.6                                      | HUVEC TNF alpha + IL4                       | 8.4                                      |
| Secondary Th2 rest             | 1.7                                      | HUVEC IL-11                                 | 3.5                                      |
| Secondary Tr1 rest             | 1.7                                      | Lung Microvascular EC none                  | 13.0                                     |
| Primary Th1 act                | 14.0                                     | Lung Microvascular EC TNFalpha + IL-1beta   | 15.3                                     |
| Primary Th2 act                | 7.7                                      | Microvascular Dermal EC none                | 23.2                                     |
| Primary Tr1 act                | 12.9                                     | Microvascular Dermal EC TNFalpha + IL-1beta | 17.3                                     |
| Primary Th1 rest               | 3.3                                      | Bronchial epithelium TNFalpha + IL1beta     | 4.5                                      |
| Primary Th2 rest               | 2.3                                      | Small airway epithelium none                | 16.0                                     |
| Primary Tr1 rest               | 2.0                                      | Small airway epithelium TNFalpha + IL-1beta | 100.0                                    |
| CD45RA CD4 lymphocyte act      | 6.5                                      | Coronary artery SMC rest                    | 15.7                                     |
| CD45RO CD4 lymphocyte act      | 5.3                                      | Coronary artery SMC TNFalpha + IL-1beta     | 11.1                                     |
| CD8 lymphocyte act             | 3.3                                      | Astrocytes rest                             | 25.3                                     |
| Secondary CD8 lymphocyte rest  | 7.2                                      | Astrocytes TNFalpha + IL-1beta              | 21.6                                     |
| Secondary CD8 lymphocyte act   | 3.0                                      | KU-812 (Basophil) rest                      | 8.4                                      |
| CD4 lymphocyte none            | 1.6                                      | KU-812 (Basophil) PMA/ionomycin             | 39.5                                     |
| 2ry Th1/Th2/Tr1_anti-CD95 CH11 | 0.3                                      | CCD1106 (Keratinocytes) none                | 35.1                                     |
| LAK cells rest                 | 19.1                                     | CCD1106 (Keratinocytes) TNFalpha + IL-1beta | 5.9                                      |
| LAK cells IL-2                 | 3.1                                      | Liver cirrhosis                             | 0.9                                      |

|                              |      |                                       |      |
|------------------------------|------|---------------------------------------|------|
| LAK cells IL-2+IL-12         | 6.5  | Lupus kidney                          | 1.3  |
| LAK cells IL-2+IFN gamma     | 9.8  | NCI-H292 none                         | 42.3 |
| LAK cells IL-2+ IL-18        | 5.9  | NCI-H292 IL-4                         | 90.1 |
| LAK cells PMA/ionomycin      | 8.7  | NCI-H292 IL-9                         | 58.2 |
| NK Cells IL-2 rest           | 1.7  | NCI-H292 IL-13                        | 33.9 |
| Two Way MLR 3 day            | 9.3  | NCI-H292 IFN gamma                    | 30.4 |
| Two Way MLR 5 day            | 7.4  | HPAEC none                            | 5.8  |
| Two Way MLR 7 day            | 2.0  | HPAEC TNF alpha + IL-1 beta           | 12.9 |
| PBMC rest                    | 1.7  | Lung fibroblast none                  | 23.8 |
| PBMC PWM                     | 12.5 | Lung fibroblast TNF alpha + IL-1 beta | 10.7 |
| PBMC PHA-L                   | 5.4  | Lung fibroblast IL-4                  | 59.0 |
| Ramos (B cell) none          | 0.5  | Lung fibroblast IL-9                  | 40.6 |
| Ramos (B cell) ionomycin     | 0.9  | Lung fibroblast IL-13                 | 31.0 |
| B lymphocytes PWM            | 15.6 | Lung fibroblast IFN gamma             | 65.5 |
| B lymphocytes CD40L and IL-4 | 5.8  | Dermal fibroblast CCD1070 rest        | 37.4 |
| EOL-1 dbcAMP                 | 3.5  | Dermal fibroblast CCD1070 TNF alpha   | 50.0 |
| EOL-1 dbcAMP PMA/ionomycin   | 60.3 | Dermal fibroblast CCD1070 IL-1 beta   | 19.6 |
| Dendritic cells none         | 17.6 | Dermal fibroblast IFN gamma           | 15.0 |
| Dendritic cells LPS          | 32.5 | Dermal fibroblast IL-4                | 43.8 |
| Dendritic cells anti-CD40    | 21.0 | IBD Colitis 2                         | 0.3  |
| Monocytes rest               | 0.1  | IBD Crohn's                           | 0.8  |
| Monocytes LPS                | 8.4  | Colon                                 | 5.3  |
| Macrophages rest             | 34.2 | Lung                                  | 15.0 |
| Macrophages LPS              | 11.3 | Thymus                                | 5.8  |
| HUVEC none                   | 6.5  | Kidney                                | 11.4 |
| HUVEC starved                | 9.3  |                                       |      |

**Panel 1.2 Summary:** Ag1508 The expression of the NOV6 gene is highest in a sample derived from skeletal muscle (CT = 19.5). Thus, this gene could be used to distinguish skeletal muscle from other tissues. Expression of the NOV6 gene is also high in kidney (CT = 23).

- 5 The NOV6 gene is also moderately expressed in other metabolically relevant tissues including heart, adrenal gland, pancreas, thyroid, pituitary gland, and liver (CT values from 29-32). The widespread expression of the NOV6 gene in tissues with metabolic function suggests a role in metabolic disorders such as obesity and diabetes.

The NOV6 gene is moderately expressed in the brain in at least the thalamus, hippocampus, cerebellum, amygdala and is highly expressed in the cerebral cortex, suggesting that this gene product has functional significance in the CNS. Please see Panel 1.3D for potential utility of this gene in the central nervous system.

5       **Panel 1.3D Summary:** Ag1586/2011/Ag2284 Two experiments with the same probe and primer set produce results that are in excellent agreement. The NOV6 gene appears to be expressed largely in cancer cell lines, with highest expression in a melanoma cell line (CTs=26-28). Of note is the expression associated with colon cancer cell lines and melanoma cell lines. Thus, the expression of this gene could be used to distinguish these samples from  
10 other samples on the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be of use in the treatment of colon cancer or melanoma.

The NOV6 gene is modestly expressed (CT values = 31-34) in a variety of metabolic tissues including pancreas, adrenal, thyroid, pituitary, fetal liver, and adipose. Thus, this gene  
15 product may be an antibody target for the treatment of metabolic disease, including obesity and diabetes, in any or all of these tissues. Furthermore, the NOV6 is expressed at higher levels in fetal (CT values = 26-28) versus adult heart (CT values = 31-33), and in fetal (CT values = 26-28) versus adult skeletal muscle (CT values = 32-33), and may be used to differentiate between the adult and fetal sources of these tissues. Furthermore, the higher  
20 levels of expression in the fetal tissues suggest that the NOV6 gene product may be involved in the development of heart and skeletal muscle tissue. Thus, therapeutic modulation of the expression or function of the protein encoded by the NOV6 gene may be beneficial in the treatment of diseases that result in weak or dystrophic heart or skeletal muscle tissue, including cardiomyopathy, atherosclerosis, hypertension, congenital heart defects, aortic  
25 stenosis, atrial septal defect (ASD), atrioventricular (A-V) canal defect, ductus arteriosus, pulmonary stenosis, subaortic stenosis, ventricular septal defect (VSD), valve diseases, muscular dystrophy, Lesch-Nyhan syndrome, and myasthenia gravis.

This gene represents a novel protein with homology to a plexin that is expressed at moderate to high levels in all brain regions examined. Plexins act as receptors for semaphorins  
30 in the CNS. The interactions of the semaphorins and their receptors are critical for axon guidance. Therefore, this gene product may be useful as a drug target in clinical conditions where axonal growth and/or compensatory synaptogenesis are desirable (spinal cord or head trauma, stroke, or neurodegenerative diseases such as Alzheimer's, Parkinson's, or Huntington's disease).

## References:

1. Pasterkamp RJ, Ruitenberg MJ, Verhaagen J. Semaphorins and their receptors in olfactory axon guidance. *Cell Mol Biol (Noisy-le-grand)* 1999 Sep;45(6):763-79

The mammalian olfactory system is capable of discriminating among a large variety of odor molecules and is therefore essential for the identification of food, enemies and mating partners. The assembly and maintenance of olfactory connectivity have been shown to depend on the combinatorial actions of a variety of molecular signals, including extracellular matrix, cell adhesion and odorant receptor molecules. Recent studies have identified semaphorins and their receptors as putative molecular cues involved in olfactory pathfinding, plasticity and regeneration. The semaphorins comprise a large family of secreted and transmembrane axon guidance proteins, being either repulsive or attractive in nature. Neuropilins were shown to serve as receptors for secreted class 3 semaphorins, whereas members of the plexin family are receptors for class 1 and V (viral) semaphorins. The present review will discuss a role for semaphorins and their receptors in the establishment and maintenance of olfactory connectivity.

2. Murakami Y, Suto F, Shimizu M, Shinoda T, Kameyama T, Fujisawa H. Differential expression of plexin-A subfamily members in the mouse nervous system. *Dev Dyn* 2001 Mar;220(3):246-58

Plexins comprise a family of transmembrane proteins (the plexin family) which are expressed in nervous tissues. Some plexins have been shown to interact directly with secreted or transmembrane semaphorins, while plexins belonging to the A subfamily are suggested to make complexes with other membrane proteins, neuropilins, and propagate chemorepulsive signals of secreted semaphorins of class 3 into cells or neurons. Despite that much information has been gathered on the plexin-semaphorin interaction, the role of plexins in the nervous system is not well understood. To gain insight into the functions of plexins in the nervous system, we analyzed spatial and temporal expression patterns of three members of the plexin-A subfamily (plexin-A1, -A2, and -A3) in the developing mouse nervous system by in situ hybridization analysis in combination with immunohistochemistry. We show that the three plexins are differentially expressed in sensory receptors or neurons in a developmentally regulated manner, suggesting that a particular plexin or set of plexins is shared by neuronal elements and functions as the receptor for semaphorins to regulate neuronal development.

**Panel 2.2 Summary:** Ag2011 The expression of this gene appears to be highest in a sample derived from a melanoma metastasis. In addition, there is substantial expression in another melanoma sample. This expression is concordant with the expression detected in Panel

1.3D. Thus, the expression of this gene could be used to distinguish melanoma from other cancer types in this panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be of use in the treatment of melanoma.

5       **Panel 2D Summary:** Ag1508/Ag1586 Expression of the SC126413398\_A gene in this panel is highest in a sample of muscle tissue adjacent to a metastatic cancer and in a metastasis of lung cancer.

10       **Panel 4.1D Summary:** Ag2284 Significant expression in this panel is limited to kidney. This observation is consistent with what was observed in other panels. Therefore, therapeutic drugs designed against the SC126413398\_A gene product may be important for regulating the function of the kidney.

15       **Panel 4D Summary:** Ag2011 Significant expression of this transcript is found in small airway epithelium upon treatment with the pro-inflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  (CT= 26.5), the muco-epidermoid cell line H 292 treated with IL-4 or IL-9, and in lung fibroblasts treated with IFN- $\gamma$  or IL-4. The constitutive expression of this transcript in these tissues is highly up-regulated by pro-inflammatory cytokines or in conditions reflecting a Th2-mediated mechanism. Therefore, modulation of the expression of the protein encoded by this transcript could be useful for the treatment of lung inflammatory diseases that result from infection of the lung (bronchitis, pneumonia) and for the treatment of Th2-mediated lung disease such as asthma or COPD. Significant expression of this transcript is also found in eosinophils upon PMA and ionomycin treatment, conditions that lead to production of eosinophil specific mediators. This production could contribute to the pathologies associated with asthma, other atopic diseases and inflammatory bowel disease. This gene encodes a novel protein with homology to members of the plexin family, a family of transmembrane proteins which act as receptors for semaphorins. In neurons, semaphorins provide essential attractive and repulsive cues that are necessary for axon guidance. The description of the interaction of plexin with tyrosine kinase in the fetal lung suggests that this protein may play a role not only in morphogenesis but also in proliferation and activation. (See reference below.) Therefore, modulation of the expression of this protein by either antibody or small molecules could be beneficial for the treatment of inflammatory lung, bowel and skin diseases.

Reference:

1. Cell 1999 Oct 1;99(1):71-80

Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates.

Tamagnone L, Artigiani S, Chen H, He Z, Ming GI, Song H, Chedotal A, Winberg ML, Goodman CS, Poo M, Tessier-Lavigne M, Comoglio PM.

Institute for Cancer Research and Treatment, University of Torino, Candiolo, Italy.  
Itamagnone@ircc.unito.it

5 In *Drosophila*, plexin A is a functional receptor for semaphorin-1a. Here we show that the human plexin gene family comprises at least nine members in four subfamilies. Plexin-B1 is a receptor for the transmembrane semaphorin Sema4D (CD100), and plexin-C1 is a receptor for the GPI-anchored semaphorin Sema7A (Sema-K1). Secreted (class 3) semaphorins do not bind directly to plexins, but rather plexins associate with neuropilins, coreceptors for these  
10 semaphorins. Plexins are widely expressed: in neurons, the expression of a truncated plexin-A1 protein blocks axon repulsion by Sema3A. The cytoplasmic domain of plexins associates with a tyrosine kinase activity. Plexins may also act as ligands mediating repulsion in epithelial cells in vitro. We conclude that plexins are receptors for multiple (and perhaps all) classes of semaphorins, either alone or in combination with neuropilins, and trigger a novel  
15 signal transduction pathway controlling cell repulsion

PMID: 10520995

## NOV7

Expression of gene NOV7 was assessed using the primer-probe sets Ag2262 and Ag2316, described in Tables 55 and 56. Results of the RTQ-PCR runs are shown in Tables 57,  
20 58 and 59.

**Table 55. Probe Name Ag2262**

| Primers | Sequences                                       | Length | Start Position | SEQ ID NO: |
|---------|-------------------------------------------------|--------|----------------|------------|
| Forward | 5'-gacctggtgtacatggagga-3'                      | 20     | 761            | 172        |
| Probe   | TET-5'-<br>cttctgccggcccagcaagtact-3'-<br>TAMRA | 23     | 790            | 173        |
| Reverse | 5'-gagcacaccctacctgctg-3'                       | 19     | 822            | 174        |

**Table 56. Probe Name Ag2316**

| Primers | Sequences                                        | Length | Start Position | SEQ ID NO: |
|---------|--------------------------------------------------|--------|----------------|------------|
| Forward | 5'-gtccaagagaggaaacaagga-3'                      | 21     | 457            | 175        |
| Probe   | TET-5'-<br>cacaatacccacgtgggcatcaag-3'-<br>TAMRA | 24     | 500            | 176        |
| Reverse | 5'-gtcctgaggccactcttcac-3'                       | 20     | 527            | 177        |



Table 57. Panel 1.3D

| Tissue Name                    | Rel.<br>Exp.(%)<br>Ag2262,<br>Run<br>150719071 | Rel.<br>Exp.(%)<br>Ag2262,<br>Run<br>167966858 | Rel.<br>Exp.(%)<br>Ag2316,<br>Run<br>162185396 | Tissue<br>Name                          | Rel.<br>Exp.(%)<br>Ag2262,<br>Run<br>150719071 | Rel.<br>Exp.(%)<br>Ag2262,<br>Run<br>167966858 | Rel.<br>Exp.(%)<br>Ag2316,<br>Run<br>162185396 |
|--------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|-----------------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|
| Liver<br>adenocarcinoma        | 0.0                                            | 6.7                                            | 0.0                                            | Kidney<br>(fetal)                       | 24.0                                           | 100.0                                          | 50.0                                           |
| Pancreas                       | 0.0                                            | 0.0                                            | 0.0                                            | Renal ca.<br>786-0                      | 0.0                                            | 0.0                                            | 0.0                                            |
| Pancreatic ca.<br>CAPAN 2      | 0.0                                            | 0.0                                            | 0.0                                            | Renal ca.<br>A498                       | 0.0                                            | 12.6                                           | 0.0                                            |
| Adrenal gland                  | 1.9                                            | 0.0                                            | 0.0                                            | Renal ca.<br>RXF 393                    | 0.0                                            | 0.0                                            | 0.0                                            |
| Thyroid                        | 2.2                                            | 0.0                                            | 0.0                                            | Renal ca.<br>ACHN                       | 0.0                                            | 0.0                                            | 0.0                                            |
| Salivary gland                 | 0.3                                            | 0.0                                            | 0.0                                            | Renal ca.<br>UO-31                      | 0.2                                            | 0.0                                            | 0.0                                            |
| Pituitary gland                | 0.0                                            | 8.0                                            | 0.0                                            | Renal ca.<br>TK-10                      | 0.0                                            | 0.0                                            | 0.0                                            |
| Brain (fetal)                  | 0.0                                            | 1.0                                            | 0.0                                            | Liver                                   | 0.0                                            | 0.0                                            | 0.0                                            |
| Brain (whole)                  | 5.2                                            | 0.0                                            | 26.2                                           | Liver (fetal)                           | 0.0                                            | 0.0                                            | 0.0                                            |
| Brain<br>(amygdala)            | 6.8                                            | 3.8                                            | 11.5                                           | Liver ca.<br>(hepatoblast)<br>HepG2     | 0.0                                            | 0.0                                            | 0.0                                            |
| Brain<br>(cerebellum)          | 1.0                                            | 6.4                                            | 0.0                                            | Lung                                    | 6.8                                            | 0.0                                            | 19.3                                           |
| Brain<br>(hippocampus)         | 16.5                                           | 0.0                                            | 0.0                                            | Lung (fetal)                            | 8.5                                            | 0.0                                            | 6.8                                            |
| Brain<br>(substantia<br>nigra) | 2.0                                            | 0.0                                            | 0.0                                            | Lung ca.<br>(small cell)<br>LX-1        | 0.0                                            | 0.0                                            | 0.0                                            |
| Brain<br>(thalamus)            | 4.9                                            | 11.2                                           | 57.0                                           | Lung ca.<br>(small cell)<br>NCI-H69     | 0.3                                            | 0.0                                            | 0.0                                            |
| Cerebral Cortex                | 2.5                                            | 13.3                                           | 3.3                                            | Lung ca.<br>(s.cell var.)<br>SHP-77     | 2.5                                            | 6.9                                            | 0.0                                            |
| Spinal cord                    | 3.3                                            | 9.2                                            | 6.8                                            | Lung ca.<br>(large<br>cell)NCI-<br>H460 | 0.0                                            | 0.0                                            | 0.0                                            |
| glio/astro U87-<br>MG          | 0.0                                            | 0.0                                            | 0.0                                            | Lung ca.<br>(non-sm.<br>cell) A549      | 0.0                                            | 6.4                                            | 0.0                                            |
| glio/astro U-<br>118-MG        | 0.0                                            | 0.0                                            | 0.0                                            | Lung ca.<br>(non-s.cell)<br>NCI-H23     | 0.0                                            | 0.0                                            | 0.0                                            |
| astrocytoma<br>SW1783          | 0.0                                            | 0.0                                            | 0.0                                            | Lung ca.<br>(non-s.cell)                | 0.0                                            | 0.0                                            | 0.0                                            |

|                                    |       |      |       |                                          |     |     |     |
|------------------------------------|-------|------|-------|------------------------------------------|-----|-----|-----|
|                                    |       |      |       | HOP-62                                   |     |     |     |
| neuro*; met<br>SK-N-AS             | 0.0   | 0.0  | 0.0   | Lung ca.<br>(non-s.cl)<br>NCI-H522       | 2.8 | 0.0 | 0.0 |
| astrocytoma SF-<br>539             | 0.0   | 0.0  | 0.0   | Lung ca.<br>(squam.) SW<br>900           | 0.0 | 0.0 | 0.0 |
| astrocytoma<br>SNB-75              | 0.0   | 0.0  | 0.0   | Lung ca.<br>(squam.)<br>NCI-H596         | 0.0 | 0.0 | 0.0 |
| glioma SNB-19                      | 0.0   | 0.0  | 0.0   | Mammary<br>gland                         | 0.0 | 0.0 | 0.0 |
| glioma U251                        | 0.0   | 0.0  | 0.0   | Breast ca.*<br>(pl.ef) MCF-<br>7         | 0.0 | 0.0 | 0.0 |
| glioma SF-295                      | 0.0   | 0.0  | 0.0   | Breast ca.*<br>(pl.ef)<br>MDA-MB-<br>231 | 0.0 | 0.0 | 0.0 |
| Heart (Fetal)                      | 2.0   | 0.0  | 33.4  | Breast ca.*<br>(pl. ef)<br>T47D          | 0.0 | 0.0 | 0.0 |
| Heart                              | 0.0   | 6.7  | 9.6   | Breast ca.<br>BT-549                     | 0.0 | 0.0 | 0.0 |
| Skeletal muscle<br>(Fetal)         | 2.5   | 0.0  | 8.2   | Breast ca.<br>MDA-N                      | 1.0 | 0.0 | 0.0 |
| Skeletal muscle                    | 0.0   | 0.0  | 0.0   | Ovary                                    | 0.0 | 0.0 | 6.4 |
| Bone marrow                        | 0.9   | 0.0  | 0.0   | Ovarian ca.<br>OVCAR-3                   | 0.0 | 0.0 | 0.0 |
| Thymus                             | 0.0   | 0.0  | 0.0   | Ovarian ca.<br>OVCAR-4                   | 0.0 | 0.0 | 0.0 |
| Spleen                             | 100.0 | 65.5 | 100.0 | Ovarian ca.<br>OVCAR-5                   | 0.0 | 0.0 | 0.0 |
| Lymph node                         | 0.0   | 0.0  | 0.0   | Ovarian ca.<br>OVCAR-8                   | 0.0 | 0.0 | 0.0 |
| Colorectal                         | 10.8  | 19.8 | 0.0   | Ovarian ca.<br>IGROV-1                   | 0.0 | 0.0 | 0.0 |
| Stomach                            | 2.7   | 0.0  | 0.0   | Ovarian ca.<br>(ascites) SK-<br>OV-3     | 0.0 | 0.0 | 0.0 |
| Small intestine                    | 6.4   | 0.0  | 0.0   | Uterus                                   | 0.0 | 0.0 | 0.0 |
| Colon ca.<br>SW480                 | 0.0   | 0.0  | 0.0   | Placenta                                 | 0.6 | 7.1 | 0.0 |
| Colon ca.*<br>SW620 (SW480<br>met) | 1.2   | 0.0  | 0.0   | Prostate                                 | 0.0 | 1.8 | 4.9 |
| Colon ca. HT29                     | 0.0   | 0.0  | 0.0   | Prostate ca.*<br>(bone met)<br>PC-3      | 0.0 | 0.0 | 0.0 |
| Colon ca. HCT-<br>116              | 0.0   | 0.0  | 0.0   | Testis                                   | 1.7 | 0.0 | 7.2 |

|                                        |      |      |      |                                  |     |     |     |
|----------------------------------------|------|------|------|----------------------------------|-----|-----|-----|
| Colon ca.<br>CaCo-2                    | 2.5  | 6.6  | 0.0  | Melanoma<br>Hs688(A).T           | 0.0 | 0.0 | 0.0 |
| CC Well to<br>Mod Diff<br>(ODO3866)    | 0.0  | 0.0  | 0.0  | Melanoma*<br>(met)<br>Hs688(B).T | 0.0 | 0.0 | 0.0 |
| Colon ca. HCC-<br>2998                 | 0.0  | 0.0  | 0.0  | Melanoma<br>UACC-62              | 0.0 | 0.0 | 0.0 |
| Gastric ca.<br>(liver met) NCI-<br>N87 | 0.0  | 14.7 | 0.0  | Melanoma<br>M14                  | 0.0 | 0.0 | 0.0 |
| Bladder                                | 0.0  | 6.5  | 16.2 | Melanoma<br>LOX IMVI             | 0.0 | 0.0 | 0.0 |
| Trachea                                | 5.0  | 0.0  | 6.0  | Melanoma*<br>(met) SK-<br>MEL-5  | 0.0 | 0.0 | 0.0 |
| Kidney                                 | 14.9 | 7.9  | 31.0 | Adipose                          | 0.0 | 0.0 | 7.6 |

Table 58. Panel 2D

| Tissue Name                                      | Rel. Exp.(%) Ag2262,<br>Run 150943107 | Tissue Name                        | Rel. Exp.(%) Ag2262,<br>Run 150943107 |
|--------------------------------------------------|---------------------------------------|------------------------------------|---------------------------------------|
| Normal Colon                                     | 14.2                                  | Kidney Margin<br>8120608           | 24.0                                  |
| CC Well to Mod Diff<br>(ODO3866)                 | 14.2                                  | Kidney Cancer 8120613              | 0.0                                   |
| CC Margin (ODO3866)                              | 0.0                                   | Kidney Margin<br>8120614           | 46.3                                  |
| CC Gr.2 rectosigmoid<br>(ODO3868)                | 0.0                                   | Kidney Cancer 9010320              | 0.0                                   |
| CC Margin (ODO3868)                              | 0.0                                   | Kidney Margin<br>9010321           | 16.5                                  |
| CC Mod Diff (ODO3920)                            | 0.0                                   | Normal Uterus                      | 16.4                                  |
| CC Margin (ODO3920)                              | 0.8                                   | Uterine Cancer 064011              | 0.0                                   |
| CC Gr.2 ascend colon<br>(ODO3921)                | 0.0                                   | Normal Thyroid                     | 15.6                                  |
| CC Margin (ODO3921)                              | 0.9                                   | Thyroid Cancer                     | 0.0                                   |
| CC from Partial<br>Hepatectomy (ODO4309)<br>Mets | 0.0                                   | Thyroid Cancer<br>A302152          | 6.8                                   |
| Liver Margin (ODO4309)                           | 1.1                                   | Thyroid Margin<br>A302153          | 0.0                                   |
| Colon mets to lung<br>(OD04451-01)               | 7.3                                   | Normal Breast                      | 9.3                                   |
| Lung Margin (OD04451-02)                         | 0.0                                   | Breast Cancer                      | 0.0                                   |
| Normal Prostate 6546-1                           | 18.6                                  | Breast Cancer<br>(OD04590-01)      | 4.8                                   |
| Prostate Cancer (OD04410)                        | 10.2                                  | Breast Cancer Mets<br>(OD04590-03) | 8.5                                   |
| Prostate Margin (OD04410)                        | 0.0                                   | Breast Cancer<br>Metastasis        | 0.0                                   |
| Prostate Cancer (OD04720-)                       | 0.0                                   | Breast Cancer                      | 7.2                                   |

|                                       |       |                                      |      |
|---------------------------------------|-------|--------------------------------------|------|
| 01)                                   |       |                                      |      |
| Prostate Margin (OD04720-02)          | 9.8   | Breast Cancer                        | 0.0  |
| Normal Lung                           | 22.5  | Breast Cancer 9100266                | 0.7  |
| Lung Met to Muscle (ODO4286)          | 6.1   | Breast Margin 9100265                | 0.0  |
| Muscle Margin (ODO4286)               | 0.0   | Breast Cancer A209073                | 0.0  |
| Lung Malignant Cancer (OD03126)       | 5.4   | Breast Margin A2090734               | 0.0  |
| Lung Margin (OD03126)                 | 0.0   | Normal Liver                         | 0.0  |
| Lung Cancer (OD04404)                 | 7.6   | Liver Cancer                         | 0.0  |
| Lung Margin (OD04404)                 | 3.8   | Liver Cancer 1025                    | 5.6  |
| Lung Cancer (OD04565)                 | 0.0   | Liver Cancer 1026                    | 2.4  |
| Lung Margin (OD04565)                 | 0.0   | Liver Cancer 6004-T                  | 0.0  |
| Lung Cancer (OD04237-01)              | 0.0   | Liver Tissue 6004-N                  | 8.7  |
| Lung Margin (OD04237-02)              | 6.9   | Liver Cancer 6005-T                  | 0.0  |
| Ocular Mel Met to Liver (ODO4310)     | 1.1   | Liver Tissue 6005-N                  | 0.0  |
| Liver Margin (ODO4310)                | 28.5  | Normal Bladder                       | 0.0  |
| Melanoma Metastasis                   | 0.0   | Bladder Cancer                       | 0.0  |
| Lung Margin (OD04321)                 | 0.0   | Bladder Cancer                       | 18.3 |
| Normal Kidney                         | 100.0 | Bladder Cancer (OD04718-01)          | 0.0  |
| Kidney Ca, Nuclear grade 2 (OD04338)  | 15.2  | Bladder Normal Adjacent (OD04718-03) | 0.0  |
| Kidney Margin (OD04338)               | 40.3  | Normal Ovary                         | 0.0  |
| Kidney Ca Nuclear grade 1/2 (OD04339) | 0.0   | Ovarian Cancer                       | 7.5  |
| Kidney Margin (OD04339)               | 50.0  | Ovarian Cancer (OD04768-07)          | 0.0  |
| Kidney Ca, Clear cell type (OD04340)  | 0.0   | Ovary Margin (OD04768-08)            | 0.0  |
| Kidney Margin (OD04340)               | 31.2  | Normal Stomach                       | 13.8 |
| Kidney Ca, Nuclear grade 3 (OD04348)  | 0.0   | Gastric Cancer 9060358               | 0.0  |
| Kidney Margin (OD04348)               | 29.9  | Stomach Margin 9060359               | 0.0  |
| Kidney Cancer (OD04622-01)            | 0.0   | Gastric Cancer 9060395               | 0.0  |
| Kidney Margin (OD04622-03)            | 58.6  | Stomach Margin 9060394               | 0.0  |
| Kidney Cancer (OD04450-01)            | 0.0   | Gastric Cancer 9060397               | 0.0  |
| Kidney Margin (OD04450-03)            | 95.9  | Stomach Margin 9060396               | 0.0  |
| Kidney Cancer 8120607                 | 0.0   | Gastric Cancer 064005                | 0.0  |

Table 59. Panel 4D

| Tissue Name                           | Rel. Exp.(%)<br>Ag2262, Run<br>150981162 | Rel. Exp.(%)<br>Ag2316, Run<br>164037437 | Tissue Name                                           | Rel. Exp.(%)<br>Ag2262, Run<br>150981162 | Rel. Exp.(%)<br>Ag2316, Run<br>164037437 |
|---------------------------------------|------------------------------------------|------------------------------------------|-------------------------------------------------------|------------------------------------------|------------------------------------------|
| Secondary Th1 act                     | 0.0                                      | 0.0                                      | HUVEC IL-1beta                                        | 0.0                                      | 0.0                                      |
| Secondary Th2 act                     | 0.0                                      | 0.0                                      | HUVEC IFN<br>gamma                                    | 0.0                                      | 0.0                                      |
| Secondary Tr1 act                     | 0.0                                      | 0.0                                      | HUVEC TNF alpha<br>+ IFN gamma                        | 11.6                                     | 0.0                                      |
| Secondary Th1 rest                    | 0.0                                      | 0.0                                      | HUVEC TNF alpha<br>+ IL4                              | 0.0                                      | 0.0                                      |
| Secondary Th2 rest                    | 0.0                                      | 0.0                                      | HUVEC IL-11                                           | 8.7                                      | 0.0                                      |
| Secondary Tr1 rest                    | 0.0                                      | 0.0                                      | Lung Microvascular<br>EC none                         | 0.0                                      | 0.0                                      |
| Primary Th1 act                       | 0.0                                      | 0.0                                      | Lung Microvascular<br>EC TNFalpha + IL-<br>1beta      | 0.0                                      | 0.0                                      |
| Primary Th2 act                       | 0.0                                      | 0.0                                      | Microvascular<br>Dermal EC none                       | 0.0                                      | 0.0                                      |
| Primary Tr1 act                       | 1.8                                      | 0.0                                      | Microvascular<br>Dermal EC<br>TNFalpha + IL-<br>1beta | 0.0                                      | 0.0                                      |
| Primary Th1 rest                      | 0.0                                      | 0.0                                      | Bronchial<br>epithelium<br>TNFalpha +<br>IL1beta      | 0.0                                      | 0.0                                      |
| Primary Th2 rest                      | 0.0                                      | 0.0                                      | Small airway<br>epithelium none                       | 0.0                                      | 0.0                                      |
| Primary Tr1 rest                      | 0.0                                      | 0.0                                      | Small airway<br>epithelium<br>TNFalpha + IL-<br>1beta | 0.0                                      | 0.0                                      |
| CD45RA CD4<br>lymphocyte act          | 0.0                                      | 0.0                                      | Coronary artery<br>SMC rest                           | 0.0                                      | 0.0                                      |
| CD45RO CD4<br>lymphocyte act          | 0.0                                      | 0.0                                      | Coronary artery<br>SMC TNFalpha +<br>IL-1beta         | 0.0                                      | 0.0                                      |
| CD8 lymphocyte act                    | 0.0                                      | 0.0                                      | Astrocytes rest                                       | 0.0                                      | 0.0                                      |
| Secondary CD8<br>lymphocyte rest      | 0.0                                      | 0.0                                      | Astrocytes<br>TNFalpha + IL-<br>1beta                 | 0.0                                      | 0.0                                      |
| Secondary CD8<br>lymphocyte act       | 0.0                                      | 0.0                                      | KU-812 (Basophil)<br>rest                             | 0.0                                      | 25.3                                     |
| CD4 lymphocyte<br>none                | 0.0                                      | 0.0                                      | KU-812 (Basophil)<br>PMA/ionomycin                    | 0.0                                      | 0.0                                      |
| 2ry<br>Th1/Th2/Tr1_anti-<br>CD95 CH11 | 0.0                                      | 0.0                                      | CCD1106<br>(Keratinocytes)<br>none                    | 0.0                                      | 0.0                                      |
| LAK cells rest                        | 0.0                                      | 0.0                                      | CCD1106                                               | 0.0                                      | 0.0                                      |

|                              |      |     |                                        |       |       |
|------------------------------|------|-----|----------------------------------------|-------|-------|
|                              |      |     | (Keratinocytes)<br>TNFalpha + IL-1beta |       |       |
| LAK cells IL-2               | 0.0  | 0.0 | Liver cirrhosis                        | 0.0   | 0.0   |
| LAK cells IL-2+IL-12         | 0.0  | 0.0 | Lupus kidney                           | 0.0   | 21.9  |
| LAK cells IL-2+IFN gamma     | 17.3 | 0.0 | NCI-H292 none                          | 0.0   | 0.0   |
| LAK cells IL-2+ IL-18        | 0.0  | 0.0 | NCI-H292 IL-4                          | 0.0   | 0.0   |
| LAK cells PMA/ionomycin      | 0.0  | 0.0 | NCI-H292 IL-9                          | 0.0   | 0.0   |
| NK Cells IL-2 rest           | 0.0  | 0.0 | NCI-H292 IL-13                         | 0.0   | 0.0   |
| Two Way MLR 3 day            | 0.0  | 0.0 | NCI-H292 IFN gamma                     | 0.0   | 0.0   |
| Two Way MLR 5 day            | 17.1 | 0.0 | HPAEC none                             | 0.0   | 0.0   |
| Two Way MLR 7 day            | 0.0  | 0.0 | HPAEC TNF alpha + IL-1 beta            | 1.3   | 0.0   |
| PBMC rest                    | 0.0  | 0.0 | Lung fibroblast none                   | 0.0   | 0.0   |
| PBMC PWM                     | 0.0  | 0.0 | Lung fibroblast TNF alpha + IL-1 beta  | 0.0   | 0.0   |
| PBMC PHA-L                   | 0.0  | 0.0 | Lung fibroblast IL-4                   | 0.0   | 0.0   |
| Ramos (B cell) none          | 0.0  | 0.0 | Lung fibroblast IL-9                   | 0.0   | 0.0   |
| Ramos (B cell) ionomycin     | 0.0  | 0.0 | Lung fibroblast IL-13                  | 0.0   | 0.0   |
| B lymphocytes PWM            | 0.0  | 0.0 | Lung fibroblast IFN gamma              | 0.0   | 0.0   |
| B lymphocytes CD40L and IL-4 | 0.0  | 0.0 | Dermal fibroblast CCD1070 rest         | 0.0   | 0.0   |
| EOL-1 dbcAMP                 | 0.0  | 0.0 | Dermal fibroblast CCD1070 TNF alpha    | 0.0   | 0.0   |
| EOL-1 dbcAMP PMA/ionomycin   | 0.0  | 0.0 | Dermal fibroblast CCD1070 IL-1 beta    | 0.0   | 0.0   |
| Dendritic cells none         | 0.0  | 0.0 | Dermal fibroblast IFN gamma            | 0.0   | 0.0   |
| Dendritic cells LPS          | 2.9  | 0.0 | Dermal fibroblast IL-4                 | 0.0   | 0.0   |
| Dendritic cells anti-CD40    | 0.0  | 0.0 | IBD Colitis 2                          | 0.0   | 0.0   |
| Monocytes rest               | 0.0  | 0.0 | IBD Crohn's                            | 0.0   | 0.0   |
| Monocytes LPS                | 0.0  | 0.0 | Colon                                  | 100.0 | 12.7  |
| Macrophages rest             | 8.2  | 0.0 | Lung                                   | 72.2  | 0.0   |
| Macrophages LPS              | 0.0  | 0.0 | Thymus                                 | 47.3  | 100.0 |
| HUVEC none                   | 0.0  | 0.0 | Kidney                                 | 0.0   | 0.0   |
| HUVEC starved                | 1.8  | 0.0 |                                        |       |       |

**CNS\_neurodegeneration\_v1.0 Summary:** Ag2316 Data from this one run is not included due to a potential problem in one of the sample wells.

**Panel 1.3D Summary:** Ag2262/2316 The expression of this gene was assessed in 3 separate runs using two independent probe and primer sets with significant expression detected in spleen and fetal kidney in all runs. Thus, the expression of this gene could be used to distinguish spleen from other tissues in the panel. Moreover, the expression of this gene could also be used to distinguish fetal kidney tissue from adult kidney tissue.

**Panel 2D Summary:** Ag2262 The expression of this gene is highest in a sample derived from normal kidney tissue. Of note was the profound association of the expression of this gene with normal kidney tissue when compared to adjacent malignant tissue. Thus, the expression of this gene could be used to distinguish normal kidney tissue from malignant kidney tissue. Moreover, therapeutic modulation of the expression or function of this gene through the use of small molecule drugs, antibodies or protein therapeutics might be of benefit in the treatment of kidney cancer.

**Panel 4D Summary:** Ag2316 This transcript is expressed almost exclusively in the thymus (CT 33.2). Therefore, this transcript could be used for detection of thymic tissues.

Ag 2262 Using a second set of primers, expression of the NOV7 gene is also found in colon and lung, in addition to its expression in the thymus. Thus, this putative Wnt -15 protein may also play an important role in the normal homeostasis of these tissues. Therefore, therapeutics designed with the protein encoded by this transcript could be important for maintaining or restoring normal function to these organs during inflammation.

## NOV8

Expression of gene NOV8 was assessed using the primer-probe set Ag2261, described in Table 60. Results of the RTQ-PCR runs are shown in Tables 61, 62 and 63.

**Table 60. Probe Name Ag2261**

| Primers | Sequences                               | Length | Start Position | SEQ ID NO: |
|---------|-----------------------------------------|--------|----------------|------------|
| Forward | 5'-ggatgactcgcttagcttct-3'              | 20     | 858            | 178        |
| Probe   | TET-5'-gccgtaggtgccaccgtgagaag-3'-TAMRA | 23     | 911            | 179        |
| Reverse | 5'-agcagatgctctcgagtt-3'                | 19     | 934            | 180        |

Table 61. Panel 1.3D

| Tissue Name                 | Rel. Exp.(%)<br>Ag2261, Run<br>150631675 | Rel. Exp.(%)<br>Ag2261, Run<br>152887692 | Tissue Name                         | Rel. Exp.(%)<br>Ag2261, Run<br>150631675 | Rel. Exp.(%)<br>Ag2261, Run<br>152887692 |
|-----------------------------|------------------------------------------|------------------------------------------|-------------------------------------|------------------------------------------|------------------------------------------|
| Liver<br>adenocarcinoma     | 22.4                                     | 19.6                                     | Kidney (fetal)                      | 2.1                                      | 0.0                                      |
| Pancreas                    | 3.9                                      | 2.5                                      | Renal ca. 786-0                     | 0.0                                      | 0.0                                      |
| Pancreatic ca.<br>CAPAN 2   | 5.3                                      | 3.5                                      | Renal ca. A498                      | 10.2                                     | 5.3                                      |
| Adrenal gland               | 2.1                                      | 0.6                                      | Renal ca. RXF<br>393                | 0.0                                      | 0.0                                      |
| Thyroid                     | 7.0                                      | 9.8                                      | Renal ca.<br>ACHN                   | 0.0                                      | 2.2                                      |
| Salivary gland              | 1.9                                      | 2.1                                      | Renal ca. UO-<br>31                 | 0.0                                      | 0.0                                      |
| Pituitary gland             | 1.0                                      | 2.2                                      | Renal ca. TK-<br>10                 | 0.0                                      | 0.0                                      |
| Brain (fetal)               | 6.8                                      | 4.9                                      | Liver                               | 0.0                                      | 0.0                                      |
| Brain (whole)               | 4.8                                      | 3.0                                      | Liver (fetal)                       | 7.6                                      | 0.0                                      |
| Brain (amygdala)            | 4.6                                      | 5.3                                      | Liver ca.<br>(hepatoblast)<br>HepG2 | 0.0                                      | 0.0                                      |
| Brain (cerebellum)          | 1.6                                      | 1.6                                      | Lung                                | 14.3                                     | 15.8                                     |
| Brain<br>(hippocampus)      | 7.5                                      | 11.3                                     | Lung (fetal)                        | 15.1                                     | 15.4                                     |
| Brain (substantia<br>nigra) | 1.2                                      | 2.6                                      | Lung ca. (small<br>cell) LX-1       | 1.6                                      | 0.0                                      |
| Brain (thalamus)            | 2.5                                      | 1.7                                      | Lung ca. (small<br>cell) NCI-H69    | 29.5                                     | 19.1                                     |
| Cerebral Cortex             | 0.0                                      | 0.0                                      | Lung ca. (s.cell<br>var.) SHP-77    | 11.0                                     | 5.1                                      |
| Spinal cord                 | 1.7                                      | 2.1                                      | Lung ca. (large<br>cell) NCI-H460   | 0.0                                      | 0.0                                      |
| glio/astro U87-MG           | 0.0                                      | 0.0                                      | Lung ca. (non-<br>sm. cell) A549    | 0.0                                      | 1.2                                      |
| glio/astro U-118-<br>MG     | 55.1                                     | 50.3                                     | Lung ca. (non-<br>s.cell) NCI-H23   | 0.0                                      | 1.3                                      |
| astrocytoma<br>SW1783       | 0.0                                      | 7.5                                      | Lung ca. (non-<br>s.cell) HOP-62    | 0.0                                      | 1.7                                      |
| neuro*; met SK-N-<br>AS     | 0.0                                      | 0.0                                      | Lung ca. (non-<br>s.cl) NCI-H522    | 8.0                                      | 8.3                                      |
| astrocytoma SF-<br>539      | 1.9                                      | 4.7                                      | Lung ca.<br>(squam.) SW<br>900      | 4.0                                      | 0.0                                      |
| astrocytoma SNB-<br>75      | 2.0                                      | 4.9                                      | Lung ca.<br>(squam.) NCI-<br>H596   | 15.8                                     | 10.2                                     |
| glioma SNB-19               | 6.7                                      | 2.4                                      | Mammary<br>gland                    | 7.2                                      | 4.1                                      |



|                                    |       |       |                                       |      |      |
|------------------------------------|-------|-------|---------------------------------------|------|------|
| glioma U251                        | 2.1   | 4.5   | Breast ca.*<br>(pl.ef) MCF-7          | 1.7  | 3.4  |
| glioma SF-295                      | 10.0  | 0.6   | Breast ca.*<br>(pl.ef) MDA-<br>MB-231 | 23.2 | 19.6 |
| Heart (Fetal)                      | 11.1  | 9.9   | Breast ca.* (pl.<br>ef) T47D          | 4.3  | 5.8  |
| Heart                              | 4.9   | 6.0   | Breast ca. BT-<br>549                 | 0.0  | 4.2  |
| Skeletal muscle<br>(Fetal)         | 100.0 | 100.0 | Breast ca.<br>MDA-N                   | 0.0  | 0.0  |
| Skeletal muscle                    | 5.5   | 8.4   | Ovary                                 | 3.6  | 3.1  |
| Bone marrow                        | 0.0   | 0.0   | Ovarian ca.<br>OVCAR-3                | 1.1  | 1.0  |
| Thymus                             | 10.0  | 3.9   | Ovarian ca.<br>OVCAR-4                | 0.0  | 0.0  |
| Spleen                             | 3.8   | 4.2   | Ovarian ca.<br>OVCAR-5                | 0.0  | 0.0  |
| Lymph node                         | 5.0   | 1.1   | Ovarian ca.<br>OVCAR-8                | 1.3  | 4.3  |
| Colorectal                         | 3.4   | 5.4   | Ovarian ca.<br>IGROV-1                | 0.0  | 0.0  |
| Stomach                            | 6.0   | 15.4  | Ovarian ca.<br>(ascites) SK-<br>OV-3  | 7.5  | 16.0 |
| Small intestine                    | 15.9  | 18.7  | Uterus                                | 17.8 | 15.1 |
| Colon ca. SW480                    | 24.3  | 15.3  | Placenta                              | 4.6  | 8.2  |
| Colon ca.* SW620<br>(SW480 met)    | 0.0   | 0.0   | Prostate                              | 3.6  | 5.3  |
| Colon ca. HT29                     | 0.0   | 0.0   | Prostate ca.*<br>(bone met) PC-<br>3  | 1.7  | 1.5  |
| Colon ca. HCT-<br>116              | 3.8   | 0.6   | Testis                                | 21.9 | 14.6 |
| Colon ca. CaCo-2                   | 0.0   | 0.8   | Melanoma<br>Hs688(A).T                | 3.1  | 4.7  |
| CC Well to Mod<br>Diff (ODO3866)   | 2.3   | 0.0   | Melanoma*<br>(met)<br>Hs688(B).T      | 0.4  | 1.3  |
| Colon ca. HCC-<br>2998             | 0.0   | 0.0   | Melanoma<br>UACC-62                   | 0.0  | 0.0  |
| Gastric ca. (liver<br>met) NCI-N87 | 16.7  | 14.9  | Melanoma M14                          | 0.0  | 0.0  |
| Bladder                            | 1.6   | 3.2   | Melanoma LOX<br>IMVI                  | 0.0  | 0.0  |
| Trachea                            | 24.3  | 33.7  | Melanoma*<br>(met) SK-MEL-<br>5       | 0.0  | 2.0  |
| Kidney                             | 0.0   | 0.0   | Adipose                               | 6.7  | 7.2  |

Table 62. Panel 2D

| Tissue Name                                      | Rel. Exp.(%)<br>Ag2261, Run<br>150811744 | Rel. Exp.(%)<br>Ag2261, Run<br>152887693 | Tissue Name                           | Rel. Exp.(%)<br>Ag2261, Run<br>150811744 | Rel. Exp.(%)<br>Ag2261, Run<br>152887693 |
|--------------------------------------------------|------------------------------------------|------------------------------------------|---------------------------------------|------------------------------------------|------------------------------------------|
| Normal Colon                                     | 19.1                                     | 19.8                                     | Kidney Margin<br>8120608              | 2.4                                      | 0.0                                      |
| CC Well to Mod<br>Diff (ODO3866)                 | 0.0                                      | 5.8                                      | Kidney Cancer<br>8120613              | 14.6                                     | 7.3                                      |
| CC Margin<br>(ODO3866)                           | 19.5                                     | 12.5                                     | Kidney Margin<br>8120614              | 4.8                                      | 1.5                                      |
| CC Gr.2<br>rectosigmoid<br>(ODO3868)             | 3.8                                      | 1.4                                      | Kidney Cancer<br>9010320              | 0.0                                      | 0.0                                      |
| CC Margin<br>(ODO3868)                           | 2.6                                      | 5.1                                      | Kidney Margin<br>9010321              | 0.0                                      | 0.0                                      |
| CC Mod Diff<br>(ODO3920)                         | 6.0                                      | 2.9                                      | Normal Uterus                         | 9.7                                      | 2.8                                      |
| CC Margin<br>(ODO3920)                           | 23.8                                     | 6.4                                      | Uterine Cancer<br>064011              | 85.9                                     | 41.5                                     |
| CC Gr.2 ascend<br>colon (ODO3921)                | 9.3                                      | 2.2                                      | Normal Thyroid                        | 15.2                                     | 7.3                                      |
| CC Margin<br>(ODO3921)                           | 16.8                                     | 11.7                                     | Thyroid Cancer                        | 0.0                                      | 3.0                                      |
| CC from Partial<br>Hepatectomy<br>(ODO4309) Mets | 2.4                                      | 0.0                                      | Thyroid Cancer<br>A302152             | 1.9                                      | 1.2                                      |
| Liver Margin<br>(ODO4309)                        | 2.6                                      | 0.0                                      | Thyroid Margin<br>A302153             | 2.6                                      | 2.8                                      |
| Colon mets to lung<br>(OD04451-01)               | 7.9                                      | 4.5                                      | Normal Breast                         | 16.2                                     | 2.7                                      |
| Lung Margin<br>(OD04451-02)                      | 11.3                                     | 12.9                                     | Breast Cancer                         | 78.5                                     | 29.7                                     |
| Normal Prostate<br>6546-1                        | 6.3                                      | 2.6                                      | Breast Cancer<br>(OD04590-01)         | 37.6                                     | 23.8                                     |
| Prostate Cancer<br>(OD04410)                     | 17.8                                     | 7.3                                      | Breast Cancer<br>Mets<br>(OD04590-03) | 100.0                                    | 24.5                                     |
| Prostate Margin<br>(OD04410)                     | 10.7                                     | 7.4                                      | Breast Cancer<br>Metastasis           | 94.0                                     | 45.4                                     |
| Prostate Cancer<br>(OD04720-01)                  | 4.7                                      | 4.4                                      | Breast Cancer                         | 25.7                                     | 24.8                                     |
| Prostate Margin<br>(OD04720-02)                  | 13.9                                     | 5.6                                      | Breast Cancer                         | 23.2                                     | 7.1                                      |
| Normal Lung                                      | 36.6                                     | 14.3                                     | Breast Cancer<br>9100266              | 33.0                                     | 7.5                                      |
| Lung Met to<br>Muscle<br>(ODO4286)               | 1.0                                      | 0.0                                      | Breast Margin<br>9100265              | 7.6                                      | 7.6                                      |
| Muscle Margin<br>(ODO4286)                       | 31.0                                     | 38.2                                     | Breast Cancer<br>A209073              | 13.9                                     | 0.9                                      |

|                                       |      |       |                                      |      |      |
|---------------------------------------|------|-------|--------------------------------------|------|------|
| Lung Malignant Cancer (OD03126)       | 81.8 | 100.0 | Breast Margin A2090734               | 2.5  | 0.0  |
| Lung Margin (OD03126)                 | 35.8 | 18.2  | Normal Liver                         | 0.0  | 0.0  |
| Lung Cancer (OD04404)                 | 57.0 | 39.5  | Liver Cancer                         | 0.0  | 0.0  |
| Lung Margin (OD04404)                 | 9.4  | 11.8  | Liver Cancer 1025                    | 4.8  | 1.7  |
| Lung Cancer (OD04565)                 | 37.1 | 42.0  | Liver Cancer 1026                    | 7.1  | 0.0  |
| Lung Margin (OD04565)                 | 22.7 | 9.3   | Liver Cancer 6004-T                  | 4.8  | 0.0  |
| Lung Cancer (OD04237-01)              | 5.3  | 6.4   | Liver Tissue 6004-N                  | 4.4  | 1.8  |
| Lung Margin (OD04237-02)              | 78.5 | 32.8  | Liver Cancer 6005-T                  | 0.0  | 6.0  |
| Ocular Mel Met to Liver (ODO4310)     | 0.0  | 0.0   | Liver Tissue 6005-N                  | 0.0  | 1.8  |
| Liver Margin (ODO4310)                | 2.4  | 0.0   | Normal Bladder                       | 2.4  | 3.0  |
| Melanoma Metastasis                   | 13.0 | 0.0   | Bladder Cancer                       | 8.5  | 4.9  |
| Lung Margin (OD04321)                 | 96.6 | 50.0  | Bladder Cancer                       | 17.0 | 11.8 |
| Normal Kidney                         | 0.0  | 0.0   | Bladder Cancer (OD04718-01)          | 10.0 | 5.7  |
| Kidney Ca, Nuclear grade 2 (OD04338)  | 0.0  | 0.0   | Bladder Normal Adjacent (OD04718-03) | 19.3 | 27.5 |
| Kidney Margin (OD04338)               | 4.0  | 4.6   | Normal Ovary                         | 13.6 | 12.4 |
| Kidney Ca Nuclear grade 1/2 (OD04339) | 0.0  | 3.3   | Ovarian Cancer                       | 37.9 | 2.1  |
| Kidney Margin (OD04339)               | 18.7 | 0.0   | Ovarian Cancer (OD04768-07)          | 18.4 | 3.7  |
| Kidney Ca, Clear cell type (OD04340)  | 8.8  | 11.7  | Ovary Margin (OD04768-08)            | 28.3 | 12.2 |
| Kidney Margin (OD04340)               | 0.0  | 2.0   | Normal Stomach                       | 48.3 | 17.3 |
| Kidney Ca, Nuclear grade 3 (OD04348)  | 3.5  | 4.0   | Gastric Cancer 9060358               | 0.0  | 0.0  |
| Kidney Margin (OD04348)               | 2.0  | 1.7   | Stomach Margin 9060359               | 9.9  | 3.0  |
| Kidney Cancer (OD04622-01)            | 9.3  | 0.0   | Gastric Cancer 9060395               | 20.7 | 10.4 |

|                               |     |     |                              |      |      |
|-------------------------------|-----|-----|------------------------------|------|------|
| Kidney Margin<br>(OD04622-03) | 0.0 | 6.3 | Stomach<br>Margin<br>9060394 | 10.0 | 12.2 |
| Kidney Cancer<br>(OD04450-01) | 0.0 | 0.0 | Gastric Cancer<br>9060397    | 8.7  | 1.5  |
| Kidney Margin<br>(OD04450-03) | 0.0 | 0.0 | Stomach<br>Margin<br>9060396 | 7.5  | 6.2  |
| Kidney Cancer<br>8120607      | 0.0 | 0.7 | Gastric Cancer<br>064005     | 10.7 | 4.8  |

Table 63. Panel 4D

| Tissue Name                        | Rel. Exp.(%)<br>Ag2261, Run<br>152887762 | Tissue Name                                    | Rel. Exp.(%)<br>Ag2261, Run<br>152887762 |
|------------------------------------|------------------------------------------|------------------------------------------------|------------------------------------------|
| Secondary Th1 act                  | 0.0                                      | HUVEC IL-1beta                                 | 0.0                                      |
| Secondary Th2 act                  | 0.0                                      | HUVEC IFN gamma                                | 3.7                                      |
| Secondary Tr1 act                  | 0.0                                      | HUVEC TNF alpha + IFN<br>gamma                 | 0.0                                      |
| Secondary Th1 rest                 | 0.0                                      | HUVEC TNF alpha + IL4                          | 4.3                                      |
| Secondary Th2 rest                 | 0.0                                      | HUVEC IL-11                                    | 4.0                                      |
| Secondary Tr1 rest                 | 0.0                                      | Lung Microvascular EC none                     | 7.2                                      |
| Primary Th1 act                    | 0.0                                      | Lung Microvascular EC<br>TNFalpha + IL-1beta   | 0.0                                      |
| Primary Th2 act                    | 0.0                                      | Microvascular Dermal EC<br>none                | 8.4                                      |
| Primary Tr1 act                    | 0.0                                      | Microvascular Dermal EC<br>TNFalpha + IL-1beta | 0.0                                      |
| Primary Th1 rest                   | 0.0                                      | Bronchial epithelium<br>TNFalpha + IL1beta     | 0.0                                      |
| Primary Th2 rest                   | 0.0                                      | Small airway epithelium<br>none                | 5.9                                      |
| Primary Tr1 rest                   | 0.0                                      | Small airway epithelium<br>TNFalpha + IL-1beta | 24.3                                     |
| CD45RA CD4<br>lymphocyte act       | 0.0                                      | Coronary artery SMC rest                       | 0.0                                      |
| CD45RO CD4<br>lymphocyte act       | 0.0                                      | Coronary artery SMC<br>TNFalpha + IL-1beta     | 0.0                                      |
| CD8 lymphocyte act                 | 0.0                                      | Astrocytes rest                                | 3.3                                      |
| Secondary CD8<br>lymphocyte rest   | 0.0                                      | Astrocytes TNFalpha + IL-<br>1beta             | 0.0                                      |
| Secondary CD8<br>lymphocyte act    | 1.6                                      | KU-812 (Basophil) rest                         | 0.0                                      |
| CD4 lymphocyte none                | 0.0                                      | KU-812 (Basophil)<br>PMA/ionomycin             | 0.0                                      |
| 2ry Th1/Th2/Tr1_anti-<br>CD95 CH11 | 0.0                                      | CCD1106 (Keratinocytes)<br>none                | 47.3                                     |
| LAK cells rest                     | 3.5                                      | CCD1106 (Keratinocytes)<br>TNFalpha + IL-1beta | 9.0                                      |

|                              |      |                                       |       |
|------------------------------|------|---------------------------------------|-------|
| LAK cells IL-2               | 0.0  | Liver cirrhosis                       | 32.8  |
| LAK cells IL-2+IL-12         | 0.0  | Lupus kidney                          | 0.0   |
| LAK cells IL-2+IFN gamma     | 0.0  | NCI-H292 none                         | 3.8   |
| LAK cells IL-2+ IL-18        | 0.0  | NCI-H292 IL-4                         | 8.0   |
| LAK cells PMA/ionomycin      | 26.1 | NCI-H292 IL-9                         | 0.0   |
| NK Cells IL-2 rest           | 0.0  | NCI-H292 IL-13                        | 13.8  |
| Two Way MLR 3 day            | 0.0  | NCI-H292 IFN gamma                    | 16.2  |
| Two Way MLR 5 day            | 0.0  | HPAEC none                            | 6.7   |
| Two Way MLR 7 day            | 0.0  | HPAEC TNF alpha + IL-1 beta           | 0.0   |
| PBMC rest                    | 0.0  | Lung fibroblast none                  | 7.6   |
| PBMC PWM                     | 0.0  | Lung fibroblast TNF alpha + IL-1 beta | 3.1   |
| PBMC PHA-L                   | 0.0  | Lung fibroblast IL-4                  | 4.3   |
| Ramos (B cell) none          | 0.0  | Lung fibroblast IL-9                  | 12.7  |
| Ramos (B cell) ionomycin     | 0.0  | Lung fibroblast IL-13                 | 6.8   |
| B lymphocytes PWM            | 0.0  | Lung fibroblast IFN gamma             | 30.4  |
| B lymphocytes CD40L and IL-4 | 3.1  | Dermal fibroblast CCD1070 rest        | 0.0   |
| EOL-1 dbcAMP                 | 0.0  | Dermal fibroblast CCD1070 TNF alpha   | 5.2   |
| EOL-1 dbcAMP PMA/ionomycin   | 3.5  | Dermal fibroblast CCD1070 IL-1 beta   | 0.0   |
| Dendritic cells none         | 0.0  | Dermal fibroblast IFN gamma           | 28.5  |
| Dendritic cells LPS          | 0.0  | Dermal fibroblast IL-4                | 42.9  |
| Dendritic cells anti-CD40    | 0.0  | IBD Colitis 2                         | 2.2   |
| Monocytes rest               | 0.0  | IBD Crohn's                           | 3.1   |
| Monocytes LPS                | 0.0  | Colon                                 | 100.0 |
| Macrophages rest             | 0.0  | Lung                                  | 36.3  |
| Macrophages LPS              | 0.0  | Thymus                                | 0.0   |
| HUVEC none                   | 0.0  | Kidney                                | 4.0   |
| HUVEC starved                | 17.4 |                                       |       |

**Panel 1.3D Summary:** Ag2261 The 88091010\_EXT gene is expressed at moderate levels in a number of metabolic tissues, with highest overall expression seen in fetal skeletal muscle (CTs=30.4-31.8). The higher levels of expression in fetal skeletal muscle when compared to adult skeletal muscle suggest that the protein product encoded by the 88091010\_EXT gene may be useful in treating muscular dystrophy, Lesch-Nyhan syndrome, myasthenia gravis and other conditions that result in weak or dystrophic muscle. This gene is also expressed in adipose, thyroid and heart. Since biologic cross-talk between adipose and

thyroid is a component of some forms of obesity, this gene product may be a protein therapeutic for the treatment of metabolic disease, including obesity and Type 2 diabetes.

**Panel 2D Summary:** Ag2261 The expression of this gene was assessed in two independent runs on panel 2D. This gene is consistently expressed in samples of breast cancer, uterine cancer and lung cancer when compared to their respective normal adjacent tissue controls. Thus, the expression of this gene could be used to distinguish breast cancer, lung cancer or uterine cancer from their normal tissues. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be of use in the treatment of breast, lung or uterine cancer.

**Panel 4D Summary:** Ag 2261: This transcript is expressed at a low, but significant level in colon (CT 33.5). Low levels of expression of this transcript are also found in the lung, keratinocytes and dermal fibroblast. Thus, this transcript could be used as a marker for thymic, lung and skin tissues. The putative Wnt -14 encoded by this transcript may play an important role in the normal homeostasis of these tissues. Therefore, therapeutics designed with the protein encoded for by this transcript could be important for maintaining or restoring normal function to these organs during inflammation.

## NOV9

Expression of NOV9 was assessed using the primer-probe set Ag2303, described in Table 64. Results of the RTQ-PCR runs are shown in Tables 65 and 66.

**Table 64. Probe Name Ag2303**

| Primers | Sequences                                  | Length | Start Position | SEQ ID NO: |
|---------|--------------------------------------------|--------|----------------|------------|
| Forward | 5'-CATTGAGAGCGATAAGTTCACA-3'               | 22     | 510            | 181        |
| Probe   | TET-5'-AGAATGTGGAGCTCAACATCCACCTG-3'-TAMRA | 26     | 548            | 182        |
| Reverse | 5'-GATGCACGCTGAAGTCATTC-3'                 | 20     | 579            | 183        |

**Table 65. Panel 1.3D**

| Tissue Name            | Rel. Exp.(%)<br>Ag2303, Run<br>167985232 | Tissue Name       | Rel. Exp.(%)<br>Ag2303, Run<br>167985232 |
|------------------------|------------------------------------------|-------------------|------------------------------------------|
| Liver adenocarcinoma   | 19.1                                     | Kidney (fetal)    | 25.5                                     |
| Pancreas               | 5.1                                      | Renal ca. 786-0   | 7.4                                      |
| Pancreatic ca. CAPAN 2 | 20.0                                     | Renal ca. A498    | 6.8                                      |
| Adrenal gland          | 2.7                                      | Renal ca. RXF 393 | 15.5                                     |

|                              |       |                                |      |
|------------------------------|-------|--------------------------------|------|
| Thyroid                      | 2.3   | Renal ca. ACHN                 | 3.9  |
| Salivary gland               | 7.2   | Renal ca. UO-31                | 6.3  |
| Pituitary gland              | 5.0   | Renal ca. TK-10                | 16.4 |
| Brain (fetal)                | 31.9  | Liver                          | 6.1  |
| Brain (whole)                | 58.2  | Liver (fetal)                  | 6.7  |
| Brain (amygdala)             | 33.9  | Liver ca. (hepatoblast) HepG2  | 11.7 |
| Brain (cerebellum)           | 55.5  | Lung                           | 14.7 |
| Brain (hippocampus)          | 23.3  | Lung (fetal)                   | 11.0 |
| Brain (substantia nigra)     | 15.3  | Lung ca. (small cell) LX-1     | 36.6 |
| Brain (thalamus)             | 21.9  | Lung ca. (small cell) NCI-H69  | 15.0 |
| Cerebral Cortex              | 80.1  | Lung ca. (s.cell var.) SHP-77  | 60.7 |
| Spinal cord                  | 8.4   | Lung ca. (large cell) NCI-H460 | 5.4  |
| glio/astro U87-MG            | 12.0  | Lung ca. (non-sm. cell) A549   | 14.3 |
| glio/astro U-118-MG          | 10.8  | Lung ca. (non-s.cell) NCI-H23  | 37.4 |
| astrocytoma SW1783           | 15.5  | Lung ca. (non-s.cell) HOP-62   | 14.5 |
| neuro*; met SK-N-AS          | 7.0   | Lung ca. (non-s.cl) NCI-H522   | 15.6 |
| astrocytoma SF-539           | 9.9   | Lung ca. (squam.) SW 900       | 16.2 |
| astrocytoma SNB-75           | 15.9  | Lung ca. (squam.) NCI-H596     | 33.2 |
| glioma SNB-19                | 8.7   | Mammary gland                  | 17.6 |
| glioma U251                  | 20.7  | Breast ca.* (pl.ef) MCF-7      | 17.1 |
| glioma SF-295                | 7.9   | Breast ca.* (pl.ef) MDA-MB-231 | 6.7  |
| Heart (Fetal)                | 46.0  | Breast ca.* (pl. ef) T47D      | 29.7 |
| Heart                        | 9.8   | Breast ca. BT-549              | 4.0  |
| Skeletal muscle (Fetal)      | 30.6  | Breast ca. MDA-N               | 10.4 |
| Skeletal muscle              | 26.6  | Ovary                          | 7.9  |
| Bone marrow                  | 29.5  | Ovarian ca. OVCAR-3            | 13.3 |
| Thymus                       | 32.3  | Ovarian ca. OVCAR-4            | 14.3 |
| Spleen                       | 26.4  | Ovarian ca. OVCAR-5            | 62.4 |
| Lymph node                   | 26.2  | Ovarian ca. OVCAR-8            | 3.9  |
| Colorectal                   | 11.0  | Ovarian ca. IGROV-1            | 6.2  |
| Stomach                      | 7.9   | Ovarian ca. (ascites) SK-OV-3  | 47.0 |
| Small intestine              | 5.6   | Uterus                         | 5.0  |
| Colon ca. SW480              | 15.6  | Placenta                       | 3.2  |
| Colon ca.* SW620 (SW480 met) | 100.0 | Prostate                       | 8.0  |
| Colon ca. HT29               | 19.5  | Prostate ca.* (bone met) PC-3  | 21.5 |
| Colon ca. HCT-116            | 16.6  | Testis                         | 5.0  |
| Colon ca. CaCo-2             | 21.9  | Melanoma Hs688(A).T            | 4.3  |

|                                  |      |                               |      |
|----------------------------------|------|-------------------------------|------|
| CC Well to Mod Diff<br>(ODO3866) | 13.1 | Melanoma* (met)<br>Hs688(B).T | 3.6  |
| Colon ca. HCC-2998               | 33.9 | Melanoma UACC-62              | 7.0  |
| Gastric ca. (liver met) NCI-N87  | 18.8 | Melanoma M14                  | 5.0  |
| Bladder                          | 7.2  | Melanoma LOX IMVI             | 13.3 |
| Trachea                          | 4.0  | Melanoma* (met) SK-MEL-5      | 7.8  |
| Kidney                           | 7.6  | Adipose                       | 13.8 |

Table 66. Panel 4D

| Tissue Name                       | Rel. Exp.(%)<br>Ag2303, Run<br>151630338 | Tissue Name                                    | Rel. Exp.(%)<br>Ag2303, Run<br>151630338 |
|-----------------------------------|------------------------------------------|------------------------------------------------|------------------------------------------|
| Secondary Th1 act                 | 69.7                                     | HUVEC IL-1beta                                 | 2.8                                      |
| Secondary Th2 act                 | 51.4                                     | HUVEC IFN gamma                                | 15.7                                     |
| Secondary Tr1 act                 | 66.0                                     | HUVEC TNF alpha + IFN<br>gamma                 | 7.2                                      |
| Secondary Th1 rest                | 24.5                                     | HUVEC TNF alpha + IL4                          | 7.2                                      |
| Secondary Th2 rest                | 28.9                                     | HUVEC IL-11                                    | 5.9                                      |
| Secondary Tr1 rest                | 29.1                                     | Lung Microvascular EC none                     | 6.8                                      |
| Primary Th1 act                   | 53.2                                     | Lung Microvascular EC<br>TNFalpha + IL-1beta   | 5.4                                      |
| Primary Th2 act                   | 44.4                                     | Microvascular Dermal EC<br>none                | 10.1                                     |
| Primary Tr1 act                   | 66.0                                     | Microvascular Dermal EC<br>TNFalpha + IL-1beta | 6.7                                      |
| Primary Th1 rest                  | 89.5                                     | Bronchial epithelium<br>TNFalpha + IL1beta     | 7.2                                      |
| Primary Th2 rest                  | 66.0                                     | Small airway epithelium none                   | 4.1                                      |
| Primary Tr1 rest                  | 46.7                                     | Small airway epithelium<br>TNFalpha + IL-1beta | 20.4                                     |
| CD45RA CD4 lymphocyte act         | 36.3                                     | Coronary artery SMC rest                       | 7.7                                      |
| CD45RO CD4 lymphocyte act         | 55.5                                     | Coronary artery SMC<br>TNFalpha + IL-1beta     | 6.1                                      |
| CD8 lymphocyte act                | 56.3                                     | Astrocytes rest                                | 4.4                                      |
| Secondary CD8 lymphocyte rest     | 47.6                                     | Astrocytes TNFalpha + IL-<br>1beta             | 3.0                                      |
| Secondary CD8 lymphocyte act      | 48.0                                     | KU-812 (Basophil) rest                         | 17.3                                     |
| CD4 lymphocyte none               | 15.2                                     | KU-812 (Basophil)<br>PMA/ionomycin             | 31.2                                     |
| 2ry Th1/Th2/Tr1_anti-CD95<br>CH11 | 41.2                                     | CCD1106 (Keratinocytes)<br>none                | 11.8                                     |
| LAK cells rest                    | 34.4                                     | CCD1106 (Keratinocytes)<br>TNFalpha + IL-1beta | 9.9                                      |
| LAK cells IL-2                    | 69.3                                     | Liver cirrhosis                                | 2.0                                      |
| LAK cells IL-2+IL-12              | 55.9                                     | Lupus kidney                                   | 2.1                                      |



|                              |       |                                       |      |
|------------------------------|-------|---------------------------------------|------|
| LAK cells IL-2+IFN gamma     | 63.3  | NCI-H292 none                         | 21.0 |
| LAK cells IL-2+ IL-18        | 57.0  | NCI-H292 IL-4                         | 33.2 |
| LAK cells PMA/ionomycin      | 9.6   | NCI-H292 IL-9                         | 33.2 |
| NK Cells IL-2 rest           | 47.6  | NCI-H292 IL-13                        | 20.9 |
| Two Way MLR 3 day            | 38.7  | NCI-H292 IFN gamma                    | 25.0 |
| Two Way MLR 5 day            | 39.5  | HPAEC none                            | 8.2  |
| Two Way MLR 7 day            | 42.0  | HPAEC TNF alpha + IL-1 beta           | 8.6  |
| PBMC rest                    | 21.5  | Lung fibroblast none                  | 5.9  |
| PBMC PWM                     | 100.0 | Lung fibroblast TNF alpha + IL-1 beta | 6.4  |
| PBMC PHA-L                   | 73.7  | Lung fibroblast IL-4                  | 12.2 |
| Ramos (B cell) none          | 54.3  | Lung fibroblast IL-9                  | 9.9  |
| Ramos (B cell) ionomycin     | 78.5  | Lung fibroblast IL-13                 | 9.6  |
| B lymphocytes PWM            | 90.1  | Lung fibroblast IFN gamma             | 11.6 |
| B lymphocytes CD40L and IL-4 | 53.6  | Dermal fibroblast CCD1070 rest        | 12.5 |
| EOL-1 dbcAMP                 | 57.4  | Dermal fibroblast CCD1070 TNF alpha   | 67.8 |
| EOL-1 dbcAMP PMA/ionomycin   | 18.8  | Dermal fibroblast CCD1070 IL-1 beta   | 9.7  |
| Dendritic cells none         | 22.1  | Dermal fibroblast IFN gamma           | 5.5  |
| Dendritic cells LPS          | 15.9  | Dermal fibroblast IL-4                | 7.4  |
| Dendritic cells anti-CD40    | 22.2  | IBD Colitis 2                         | 2.0  |
| Monocytes rest               | 45.4  | IBD Crohn's                           | 1.4  |
| Monocytes LPS                | 17.3  | Colon                                 | 20.4 |
| Macrophages rest             | 36.1  | Lung                                  | 14.0 |
| Macrophages LPS              | 18.0  | Thymus                                | 10.6 |
| HUVEC none                   | 13.7  | Kidney                                | 31.6 |
| HUVEC starved                | 19.8  |                                       |      |

### Panel 1.3D Summary: Ag2303

NOV9 is widely expressed across the panel, with highest expression in a colon cancer cell line SW620 (CT=26.4). Of note is the difference in expression between the related colon cancer cell lines SW620 and SW480. SW480 represents the primary lesion from a patient with colon cancer, while SW620 represents a metastasis from the same patient. The difference in expression of this gene between the SW620 and SW480 cell lines indicates that it could be used to distinguish these cells, or others like them. Moreover, therapeutic modulation of NOV9, through the use of small molecule drugs, antibodies or protein therapeutics, may be effective in the treatment of metastatic colon cancer.

Among tissues with metabolic function, NOV9 is moderately expressed in the pancreas, adrenal, thyroid, pituitary, adipose, adult and fetal heart, and adult and fetal liver. This expression profile suggests that the NOV9 product may be an important small molecule target for the treatment of metabolic disease in any or all of these tissues, including obesity and diabetes.

NOV9, which encodes a beta-adrenergic receptor kinase, also shows high expression in all regions of the brain examined, especially in the cerebral cortex (CT=26.7) The beta adrenergic receptors have been shown to play a role in memory formation and in clinical depression. Since many current anti-depressants produce undesired side effects as a result of non-specific binding (to other receptors), this gene is therefore an excellent small molecule target for the treatment of clinical depression without side effects. Furthermore, the role of beta adrenergic receptors in memory consolidation suggests that the NOV9 gene product would also be useful as a small molecule target for the treatment of Alzheimer's disease, vascular dementia, or any memory loss disorder.

#### References:

1. Feighner JP. Mechanism of action of antidepressant medications. J Clin Psychiatry 1999;60 Suppl 4:4-11; discussion 12-3

The psychopharmacology of depression is a field that has evolved rapidly in just under 5 decades. Early antidepressant medications--tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs)--were discovered through astute clinical observations. These first-generation medications were effective because they enhanced serotonergic or noradrenergic mechanisms or both. Unfortunately, the TCAs also blocked histaminic, cholinergic, and alpha1-adrenergic receptor sites, and this action brought about unwanted side effects such as weight gain, dry mouth, constipation, drowsiness, and dizziness. MAOIs can interact with tyramine to cause potentially lethal hypertension and present potentially dangerous interactions with a number of medications and over-the-counter drugs. The newest generation of antidepressants, including the single-receptor selective serotonin reuptake inhibitors (SSRIs) and multiple-receptor antidepressants venlafaxine, mirtazapine, bupropion, trazodone, and nefazodone, target one or more specific brain receptor sites without, in most cases, activating unwanted sites such as histamine and acetylcholine. This paper discusses the new antidepressants, particularly with regard to mechanism of action, and looks at future developments in the treatment of depression.

2. Ferry B, McGaugh JL. Role of amygdala norepinephrine in mediating stress hormone regulation of memory storage. *Acta Pharmacol Sin* 2000 Jun;21(6):481-93

There is extensive evidence indicating that the noradrenergic system of the amygdala, particularly the basolateral nucleus of the amygdala (BLA), is involved in memory consolidation. This article reviews the central hypothesis that stress hormones released during emotionally arousing experiences activate noradrenergic mechanisms in the BLA, resulting in enhanced memory for those events. Findings from experiments using rats have shown that the memory-modulatory effects of the adrenocortical stress hormones epinephrine and glucocorticoids involve activation of beta-adrenoceptors in the BLA. In addition, both behavioral and microdialysis studies have shown that the noradrenergic system of the BLA also mediates the influences of other neuromodulatory systems such as opioid peptidergic and GABAergic systems on memory storage. Other findings indicate that this stress hormone-induced activation of noradrenergic mechanisms in the BLA regulates memory storage in other brain regions.

#### Panel 4D Summary: Ag2303

NOV9, a beta-adrenergic receptor kinase homolog, is highly expressed (CTs 26-29) in a wide range of cells that play a significance role in the immune response. Highest expression of this gene is found in activated B and T cells. Therefore, inhibition of the function of the protein encoded by NOV9 with a small molecule drug may block the functions of B cells or T cells and could be beneficial in the treatment of patients suffering from autoimmune and inflammatory diseases such as asthma, allergies, inflammatory bowel disease, lupus erythematosus, or rheumatoid arthritis.

#### NOV10

Expression of NOV10 was assessed using the primer-probe set Ag2311, described in Table 67. Results of the RTQ-PCR runs are shown in Tables 68, 69, 70 and 71.

**Table 67. Probe Name Ag2311**

| Primers | Sequences                                          | Length | Start Position | SEQ ID NO: |
|---------|----------------------------------------------------|--------|----------------|------------|
| Forward | 5'-CTCTGGGGACTCCTAATTCTG-3'                        | 22     | 2913           | 184        |
| Probe   | TET-5'-<br>CCCAGCCTAAAGCAGGGATCAGTCTT-3'-<br>TAMRA | 26     | 2939           | 185        |
| Reverse | 5'-TCCAAGGATTATTCCACAAGA-3'                        | 22     | 2966           | 186        |

Table 68. CNS\_neurodegeneration\_v1.0

| Tissue Name                   | Rel. Exp.(%)<br>Ag2311, Run<br>208253895 | Tissue Name                    | Rel. Exp.(%)<br>Ag2311, Run<br>208253895 |
|-------------------------------|------------------------------------------|--------------------------------|------------------------------------------|
| AD 1 Hippo                    | 33.4                                     | Control (Path) 3 Temporal Ctx  | 13.4                                     |
| AD 2 Hippo                    | 46.3                                     | Control (Path) 4 Temporal Ctx  | 44.8                                     |
| AD 3 Hippo                    | 12.9                                     | AD 1 Occipital Ctx             | 36.1                                     |
| AD 4 Hippo                    | 15.4                                     | AD 2 Occipital Ctx (Missing)   | 0.0                                      |
| AD 5 Hippo                    | 87.7                                     | AD 3 Occipital Ctx             | 10.5                                     |
| AD 6 Hippo                    | 41.2                                     | AD 4 Occipital Ctx             | 23.2                                     |
| Control 2 Hippo               | 34.4                                     | AD 5 Occipital Ctx             | 40.1                                     |
| Control 4 Hippo               | 29.7                                     | AD 5 Occipital Ctx             | 28.3                                     |
| Control (Path) 3 Hippo        | 13.0                                     | Control 1 Occipital Ctx        | 8.8                                      |
| AD 1 Temporal Ctx             | 39.2                                     | Control 2 Occipital Ctx        | 57.4                                     |
| AD 2 Temporal Ctx             | 46.7                                     | Control 3 Occipital Ctx        | 32.3                                     |
| AD 3 Temporal Ctx             | 12.2                                     | Control 4 Occipital Ctx        | 13.6                                     |
| AD 4 Temporal Ctx             | 42.9                                     | Control (Path) 1 Occipital Ctx | 67.4                                     |
| AD 5 Inf Temporal Ctx         | 100.0                                    | Control (Path) 2 Occipital Ctx | 26.8                                     |
| AD 5 Sup Temporal Ctx         | 57.8                                     | Control (Path) 3 Occipital Ctx | 12.5                                     |
| AD 6 Inf Temporal Ctx         | 48.3                                     | Control (Path) 4 Occipital Ctx | 36.6                                     |
| AD 6 Sup Temporal Ctx         | 42.6                                     | Control 1 Parietal Ctx         | 14.1                                     |
| Control 1 Temporal Ctx        | 15.7                                     | Control 2 Parietal Ctx         | 71.7                                     |
| Control 2 Temporal Ctx        | 37.4                                     | Control 3 Parietal Ctx         | 29.1                                     |
| Control 3 Temporal Ctx        | 25.5                                     | Control (Path) 1 Parietal Ctx  | 39.5                                     |
| Control 3 Temporal Ctx        | 23.5                                     | Control (Path) 2 Parietal Ctx  | 31.2                                     |
| Control (Path) 1 Temporal Ctx | 59.5                                     | Control (Path) 3 Parietal Ctx  | 11.6                                     |
| Control (Path) 2 Temporal Ctx | 35.8                                     | Control (Path) 4 Parietal Ctx  | 58.2                                     |

Table 69. Panel 1.3D

| Tissue Name            | Rel. Exp.(%)<br>Ag2311, Run<br>165627680 | Tissue Name                   | Rel. Exp.(%)<br>Ag2311, Run<br>165627680 |
|------------------------|------------------------------------------|-------------------------------|------------------------------------------|
| Liver adenocarcinoma   | 1.7                                      | Kidney (fetal)                | 6.7                                      |
| Pancreas               | 10.5                                     | Renal ca. 786-0               | 1.3                                      |
| Pancreatic ca. CAPAN 2 | 5.4                                      | Renal ca. A498                | 6.5                                      |
| Adrenal gland          | 22.2                                     | Renal ca. RXF 393             | 3.3                                      |
| Thyroid                | 21.5                                     | Renal ca. ACHN                | 0.9                                      |
| Salivary gland         | 10.6                                     | Renal ca. UO-31               | 1.1                                      |
| Pituitary gland        | 24.7                                     | Renal ca. TK-10               | 1.3                                      |
| Brain (fetal)          | 15.0                                     | Liver                         | 7.5                                      |
| Brain (whole)          | 23.7                                     | Liver (fetal)                 | 10.8                                     |
| Brain (amygdala)       | 24.7                                     | Liver ca. (hepatoblast) HepG2 | 2.0                                      |
| Brain (cerebellum)     | 24.5                                     | Lung                          | 27.5                                     |

|                                 |       |                                |      |
|---------------------------------|-------|--------------------------------|------|
| Brain (hippocampus)             | 35.1  | Lung (fetal)                   | 7.5  |
| Brain (substantia nigra)        | 100.0 | Lung ca. (small cell) LX-1     | 4.6  |
| Brain (thalamus)                | 27.7  | Lung ca. (small cell) NCI-H69  | 0.0  |
| Cerebral Cortex                 | 9.9   | Lung ca. (s.cell var.) SHP-77  | 5.4  |
| Spinal cord                     | 32.5  | Lung ca. (large cell) NCI-H460 | 19.1 |
| glio/astro U87-MG               | 4.2   | Lung ca. (non-sm. cell) A549   | 3.2  |
| glio/astro U-118-MG             | 9.2   | Lung ca. (non-s.cell) NCI-H23  | 5.1  |
| astrocytoma SW1783              | 3.7   | Lung ca. (non-s.cell) HOP-62   | 3.5  |
| neuro*; met SK-N-AS             | 12.2  | Lung ca. (non-s.cl) NCI-H522   | 1.4  |
| astrocytoma SF-539              | 4.1   | Lung ca. (squam.) SW 900       | 1.9  |
| astrocytoma SNB-75              | 3.7   | Lung ca. (squam.) NCI-H596     | 0.3  |
| glioma SNB-19                   | 5.8   | Mammary gland                  | 18.0 |
| glioma U251                     | 28.9  | Breast ca.* (pl.ef) MCF-7      | 6.1  |
| glioma SF-295                   | 4.5   | Breast ca.* (pl.ef) MDA-MB-231 | 3.8  |
| Heart (Fetal)                   | 5.4   | Breast ca.* (pl. ef) T47D      | 6.3  |
| Heart                           | 12.3  | Breast ca. BT-549              | 1.0  |
| Skeletal muscle (Fetal)         | 4.6   | Breast ca. MDA-N               | 2.1  |
| Skeletal muscle                 | 22.8  | Ovary                          | 2.0  |
| Bone marrow                     | 15.1  | Ovarian ca. OVCAR-3            | 4.3  |
| Thymus                          | 17.9  | Ovarian ca. OVCAR-4            | 1.8  |
| Spleen                          | 21.9  | Ovarian ca. OVCAR-5            | 9.8  |
| Lymph node                      | 27.7  | Ovarian ca. OVCAR-8            | 1.0  |
| Colorectal                      | 3.0   | Ovarian ca. IGROV-1            | 0.8  |
| Stomach                         | 12.9  | Ovarian ca. (ascites) SK-OV-3  | 2.8  |
| Small intestine                 | 66.4  | Uterus                         | 29.3 |
| Colon ca. SW480                 | 2.4   | Placenta                       | 8.2  |
| Colon ca.* SW620 (SW480 met)    | 4.1   | Prostate                       | 28.7 |
| Colon ca. HT29                  | 2.4   | Prostate ca.* (bone met) PC-3  | 1.7  |
| Colon ca. HCT-116               | 2.7   | Testis                         | 38.7 |
| Colon ca. CaCo-2                | 3.0   | Melanoma Hs688(A).T            | 1.3  |
| CC Well to Mod Diff (ODO3866)   | 5.8   | Melanoma* (met) Hs688(B).T     | 2.0  |
| Colon ca. HCC-2998              | 3.5   | Melanoma UACC-62               | 2.9  |
| Gastric ca. (liver met) NCI-N87 | 13.8  | Melanoma M14                   | 11.0 |
| Bladder                         | 4.5   | Melanoma LOX IMVI              | 0.2  |
| Trachea                         | 13.1  | Melanoma* (met) SK-MEL-5       | 2.9  |
| Kidney                          | 14.7  | Adipose                        | 9.7  |

Table 70. Panel 2.2

| Tissue Name                                    | Rel. Exp.(%)<br>Ag2311, Run<br>174370590 | Tissue Name                                 | Rel. Exp.(%)<br>Ag2311, Run<br>174370590 |
|------------------------------------------------|------------------------------------------|---------------------------------------------|------------------------------------------|
| Normal Colon                                   | 9.4                                      | Kidney Margin (OD04348)                     | 100.0                                    |
| Colon cancer (OD06064)                         | 1.2                                      | Kidney malignant cancer<br>(OD06204B)       | 9.3                                      |
| Colon Margin (OD06064)                         | 0.6                                      | Kidney normal adjacent tissue<br>(OD06204E) | 17.6                                     |
| Colon cancer (OD06159)                         | 1.5                                      | Kidney Cancer (OD04450-01)                  | 41.2                                     |
| Colon Margin (OD06159)                         | 5.5                                      | Kidney Margin (OD04450-03)                  | 14.9                                     |
| Colon cancer (OD06297-04)                      | 1.1                                      | Kidney Cancer 8120613                       | 2.9                                      |
| Colon Margin (OD06297-015)                     | 9.6                                      | Kidney Margin 8120614                       | 9.8                                      |
| CC Gr.2 ascend colon<br>(ODO3921)              | 9.8                                      | Kidney Cancer 9010320                       | 6.7                                      |
| CC Margin (ODO3921)                            | 3.6                                      | Kidney Margin 9010321                       | 5.4                                      |
| Colon cancer metastasis<br>(OD06104)           | 5.5                                      | Kidney Cancer 8120607                       | 6.3                                      |
| Lung Margin (OD06104)                          | 1.2                                      | Kidney Margin 8120608                       | 6.3                                      |
| Colon mets to lung (OD04451-<br>01)            | 22.2                                     | Normal Uterus                               | 17.6                                     |
| Lung Margin (OD04451-02)                       | 12.0                                     | Uterine Cancer 064011                       | 11.2                                     |
| Normal Prostate                                | 6.2                                      | Normal Thyroid                              | 3.3                                      |
| Prostate Cancer (OD04410)                      | 4.3                                      | Thyroid Cancer                              | 11.1                                     |
| Prostate Margin (OD04410)                      | 9.6                                      | Thyroid Cancer A302152                      | 33.4                                     |
| Normal Ovary                                   | 17.3                                     | Thyroid Margin A302153                      | 9.7                                      |
| Ovarian cancer (OD06283-03)                    | 6.7                                      | Normal Breast                               | 28.7                                     |
| Ovarian Margin (OD06283-07)                    | 8.8                                      | Breast Cancer                               | 9.7                                      |
| Ovarian Cancer                                 | 10.7                                     | Breast Cancer                               | 14.9                                     |
| Ovarian cancer (OD06145)                       | 4.2                                      | Breast Cancer (OD04590-01)                  | 32.5                                     |
| Ovarian Margin (OD06145)                       | 29.5                                     | Breast Cancer Mets<br>(OD04590-03)          | 12.2                                     |
| Ovarian cancer (OD06455-03)                    | 7.9                                      | Breast Cancer Metastasis                    | 25.5                                     |
| Ovarian Margin (OD06455-07)                    | 2.4                                      | Breast Cancer                               | 15.9                                     |
| Normal Lung                                    | 26.2                                     | Breast Cancer 9100266                       | 1.8                                      |
| Invasive poor diff. lung adeno<br>(ODO4945-01) | 8.3                                      | Breast Margin 9100265                       | 1.6                                      |
| Lung Margin (ODO4945-03)                       | 6.0                                      | Breast Cancer A209073                       | 2.1                                      |
| Lung Malignant Cancer<br>(OD03126)             | 16.0                                     | Breast Margin A2090734                      | 26.6                                     |
| Lung Margin (OD03126)                          | 5.5                                      | Breast cancer (OD06083)                     | 25.0                                     |
| Lung Cancer (OD05014A)                         | 7.0                                      | Breast cancer node metastasis<br>(OD06083)  | 27.5                                     |
| Lung Margin (OD05014B)                         | 7.3                                      | Normal Liver                                | 23.7                                     |

|                                       |      |                        |      |
|---------------------------------------|------|------------------------|------|
| Lung cancer (OD06081)                 | 20.4 | Liver Cancer 1026      | 2.7  |
| Lung Margin (OD06081)                 | 12.3 | Liver Cancer 1025      | 29.5 |
| Lung Cancer (OD04237-01)              | 9.5  | Liver Cancer 6004-T    | 24.0 |
| Lung Margin (OD04237-02)              | 18.4 | Liver Tissue 6004-N    | 23.2 |
| Ocular Mel Met to Liver (ODO4310)     | 14.1 | Liver Cancer 6005-T    | 9.0  |
| Liver Margin (ODO4310)                | 15.0 | Liver Tissue 6005-N    | 51.1 |
| Melanoma Metastasis                   | 12.5 | Liver Cancer           | 31.4 |
| Lung Margin (OD04321)                 | 4.4  | Normal Bladder         | 13.5 |
| Normal Kidney                         | 20.4 | Bladder Cancer         | 6.4  |
| Kidney Ca, Nuclear grade 2 (OD04338)  | 63.3 | Bladder Cancer         | 11.3 |
| Kidney Margin (OD04338)               | 11.5 | Normal Stomach         | 48.6 |
| Kidney Ca Nuclear grade 1/2 (OD04339) | 46.3 | Gastric Cancer 9060397 | 5.6  |
| Kidney Margin (OD04339)               | 17.4 | Stomach Margin 9060396 | 4.0  |
| Kidney Ca, Clear cell type (OD04340)  | 24.8 | Gastric Cancer 9060395 | 4.0  |
| Kidney Margin (OD04340)               | 17.0 | Stomach Margin 9060394 | 9.1  |
| Kidney Ca, Nuclear grade 3 (OD04348)  | 3.1  | Gastric Cancer 064005  | 7.9  |

Table 71. Panel 4D

| Tissue Name               | Rel. Exp.(%)<br>Ag2311, Run<br>158928074 | Tissue Name                                 | Rel. Exp.(%)<br>Ag2311, Run<br>158928074 |
|---------------------------|------------------------------------------|---------------------------------------------|------------------------------------------|
| Secondary Th1 act         | 29.3                                     | HUVEC IL-1beta                              | 6.1                                      |
| Secondary Th2 act         | 33.2                                     | HUVEC IFN gamma                             | 37.6                                     |
| Secondary Tr1 act         | 45.7                                     | HUVEC TNF alpha + IFN gamma                 | 36.3                                     |
| Secondary Th1 rest        | 17.3                                     | HUVEC TNF alpha + IL4                       | 34.9                                     |
| Secondary Th2 rest        | 19.2                                     | HUVEC IL-11                                 | 17.8                                     |
| Secondary Tr1 rest        | 27.7                                     | Lung Microvascular EC none                  | 30.4                                     |
| Primary Th1 act           | 36.9                                     | Lung Microvascular EC TNFalpha + IL-1beta   | 43.8                                     |
| Primary Th2 act           | 36.9                                     | Microvascular Dermal EC none                | 39.5                                     |
| Primary Tr1 act           | 45.7                                     | Microvascular Dermal EC TNFalpha + IL-1beta | 27.4                                     |
| Primary Th1 rest          | 38.7                                     | Bronchial epithelium TNFalpha + IL1beta     | 1.2                                      |
| Primary Th2 rest          | 27.0                                     | Small airway epithelium none                | 6.2                                      |
| Primary Tr1 rest          | 37.6                                     | Small airway epithelium TNFalpha + IL-1beta | 20.0                                     |
| CD45RA CD4 lymphocyte act | 17.2                                     | Coronary artery SMC rest                    | 9.5                                      |

|                                   |      |                                                |       |
|-----------------------------------|------|------------------------------------------------|-------|
| CD45RO CD4 lymphocyte act         | 24.0 | Coronary artery SMC<br>TNFalpha + IL-1beta     | 7.7   |
| CD8 lymphocyte act                | 21.9 | Astrocytes rest                                | 10.9  |
| Secondary CD8 lymphocyte rest     | 26.2 | Astrocytes TNFalpha + IL-1beta                 | 9.9   |
| Secondary CD8 lymphocyte act      | 17.4 | KU-812 (Basophil) rest                         | 40.1  |
| CD4 lymphocyte none               | 5.9  | KU-812 (Basophil)<br>PMA/ionomycin             | 52.9  |
| 2ry Th1/Th2/Tr1_anti-CD95<br>CH11 | 19.8 | CCD1106 (Keratinocytes)<br>none                | 9.6   |
| LAK cells rest                    | 32.8 | CCD1106 (Keratinocytes)<br>TNFalpha + IL-1beta | 0.6   |
| LAK cells IL-2                    | 23.3 | Liver cirrhosis                                | 10.8  |
| LAK cells IL-2+IL-12              | 31.0 | Lupus kidney                                   | 6.9   |
| LAK cells IL-2+IFN gamma          | 27.5 | NCI-H292 none                                  | 65.1  |
| LAK cells IL-2+ IL-18             | 38.7 | NCI-H292 IL-4                                  | 65.1  |
| LAK cells PMA/ionomycin           | 17.4 | NCI-H292 IL-9                                  | 71.7  |
| NK Cells IL-2 rest                | 28.5 | NCI-H292 IL-13                                 | 41.2  |
| Two Way MLR 3 day                 | 36.9 | NCI-H292 IFN gamma                             | 43.8  |
| Two Way MLR 5 day                 | 19.5 | HPAEC none                                     | 30.8  |
| Two Way MLR 7 day                 | 16.4 | HPAEC TNF alpha + IL-1<br>beta                 | 16.8  |
| PBMC rest                         | 23.5 | Lung fibroblast none                           | 25.7  |
| PBMC PWM                          | 35.1 | Lung fibroblast TNF alpha +<br>IL-1 beta       | 9.0   |
| PBMC PHA-L                        | 17.8 | Lung fibroblast IL-4                           | 43.5  |
| Ramos (B cell) none               | 21.8 | Lung fibroblast IL-9                           | 27.4  |
| Ramos (B cell) ionomycin          | 37.4 | Lung fibroblast IL-13                          | 29.1  |
| B lymphocytes PWM                 | 72.7 | Lung fibroblast IFN gamma                      | 23.5  |
| B lymphocytes CD40L and IL-4      | 66.0 | Dermal fibroblast CCD1070<br>rest              | 42.3  |
| EOL-1 dbcAMP                      | 35.8 | Dermal fibroblast CCD1070<br>TNF alpha         | 62.9  |
| EOL-1 dbcAMP<br>PMA/ionomycin     | 38.4 | Dermal fibroblast CCD1070<br>IL-1 beta         | 28.3  |
| Dendritic cells none              | 59.0 | Dermal fibroblast IFN gamma                    | 18.3  |
| Dendritic cells LPS               | 32.5 | Dermal fibroblast IL-4                         | 33.2  |
| Dendritic cells anti-CD40         | 33.4 | IBD Colitis 2                                  | 5.3   |
| Monocytes rest                    | 42.9 | IBD Crohn's                                    | 5.2   |
| Monocytes LPS                     | 25.0 | Colon                                          | 27.5  |
| Macrophages rest                  | 42.3 | Lung                                           | 14.5  |
| Macrophages LPS                   | 18.7 | Thymus                                         | 47.6  |
| HUVEC none                        | 37.9 | Kidney                                         | 100.0 |
| HUVEC starved                     | 40.1 |                                                |       |



**CNS\_neurodegeneration\_v1.0 Summary: Ag2311**

NOV10 does not show differential expression between Alzheimer's diseased brains and control brains. However, this panel confirms the expression of this gene in the brains of an independent group of patients. Please see panel 1.3d for discussion of utility in the central nervous system.

**Panel 1.3D Summary: Ag2311**

NOV10, an alpha mannosidase isoform, is expressed at moderate levels in all regions of the brain examined, with highest expression in the substantia nigra (CT=29.3). In the brain, alpha mannosidase has been implicated in the processes of myelination and axon growth.

Therefore, therapeutic modulation of this gene or its protein product may be of use in the treatment of disorders where myelination has been compromised such as multiple sclerosis, and schizophrenia. In addition, the protein encoded by NOV10 could be useful in clinical situations where increased axonal growth is desired including spinal cord or brain trauma, stroke, or peripheral nerve injury.

NOV10 gene is moderately expressed (CT values = 31-34) in a variety of metabolic tissues including pancreas, adrenal, thyroid, pituitary, adult and fetal heart, adult and fetal liver, adult and fetal skeletal muscle, and adipose. This expression profile suggests that the protein encoded by the NOV10 may be an important small molecule target for the treatment of metabolic disease in any or all of these tissues, including obesity and diabetes.

The expression of this gene appears to be generally associated with normal tissues when compared to cell lines. Of note was the difference in expression in normal prostate when compared to the prostate cancer cell line (PC-3). Thus, NOV10 could be used to distinguish this sample on the panel from other samples or to distinguish normal prostate from prostate cancer. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics, might be of use in the treatment of prostate cancer.

**References:**

1. Vite CH, McGowan JC, Braund KG, Drobatz KJ, Glickson JD, Wolfe JH, Haskins ME. Histopathology, electrodiagnostic testing, and magnetic resonance imaging show significant peripheral and central nervous system myelin abnormalities in the cat model of alpha-mannosidosis. J Neuropathol Exp Neurol 2001 Aug;60(8):817-28

Alpha-mannosidosis is a disease caused by the deficient activity of alpha-mannosidase, a lysosomal hydrolase involved in the degradation of glycoproteins. The disease is

characterized by the accumulation of mannose-rich oligosaccharides within lysosomes. The purpose of this study was to characterize the peripheral nervous system (PNS) and central nervous system (CNS) myelin abnormalities in cats from a breeding colony with a uniform mutation in the gene encoding alpha-mannosidase. Three affected cats and 3 normal cats from 2 litters were examined weekly from 4 to 18 wk of age. Progressively worsening neurological signs developed in affected cats that included tremors, loss of balance, and nystagmus. In the PNS, affected cats showed slow motor nerve conduction velocity and increased F-wave latency. Single nerve fiber teasing revealed significant demyelination/remyelination in affected cats. Mean G-ratios of nerves showed a significant increase in affected cats compared to normal cats. Magnetic resonance imaging of the CNS revealed diffuse white matter signal abnormalities throughout the brain of affected cats. Quantitative magnetization transfer imaging showed a 8%-16% decrease in the magnetization transfer ratio in brain white matter of affected cats compared to normal cats, consistent with myelin abnormalities. Histology confirmed myelin loss throughout the cerebrum and cerebellum. Thus, histology, electrodiagnostic testing, and magnetic resonance imaging identified significant myelination abnormalities in both the PNS and CNS that have not been described previously in alpha-mannosidosis.

2. Zmuda JF, Rivas RJ. The Golgi apparatus and the centrosome are localized to the sites of newly emerging axons in cerebellar granule neurons in vitro. *Cell Motil Cytoskeleton* 1998;41(1):18-38

Cultured cerebellar granule neurons develop their characteristic axonal and dendritic morphologies in a series of discrete temporal steps highly similar to those observed in situ, initially extending a single process, followed by the extension of a second process from the opposite pole of the cell, both of which develop into axons to generate a bipolar morphology. A mature morphology is attained following the outgrowth of multiple, short dendrites [Powell et al., 1997: *J. Neurobiol.* 32:223-236]. To determine the relationship between the localization of the Golgi apparatus, the site of microtubule nucleation (the centrosome), and the sites of initial and secondary axonal extension, the intracellular positioning of the Golgi and centrosome was observed during the differentiation of postnatal mouse granule neurons in vitro. The Golgi was labeled using the fluorescent lipid analogue, C5-DMB-Ceramide, or by indirect immunofluorescence using antibodies against the Golgi resident protein, alpha-mannosidase II. At 1-2 days in vitro (DIV), the Golgi was positioned at the base of the initial process in 99% of unipolar cells observed. By 3 DIV, many cells began the transition to a bipolar morphology by extending a short neurite from the pole of the cell opposite to the initial

process. The Golgi was observed at this site of secondary outgrowth in 92% of these "transitional" cells, suggesting that the Golgi was repositioned from the base of the initial process to the site of secondary neurite outgrowth. As the second process elongated and the cells proceeded to the bipolar stage of development, or at later stages when distinct axonal and somatodendritic domains had been established, the Golgi was not consistently positioned at the base of either axons or dendrites, and was most often found at sites on the plasma membrane from which no processes originated. To determine the location of the centrosome in relation to the Golgi during development, granule neurons were labeled with antibodies against gamma-tubulin and optically sectioned using confocal microscopy. The centrosome was consistently co-localized with the Golgi during all stages of differentiation, and also appeared to be repositioned to the base of the newly emerging axon during the transition from a unipolar to a bipolar morphology. These findings indicate that during the early stages of granule cell axonal outgrowth, the Golgi-centrosome is positioned at the base of the initial axon and is then repositioned to the base of the newly emerging secondary axon. Such an intracellular reorientation of these organelles may be important in maintaining the characteristic developmental pattern of granule neurons by establishing the polarized microtubule network and the directed flow of membranous vesicles required for initial axonal elaboration

#### **Panel 2.2 Summary: Ag2311**

The expression of this gene is highest in a sample derived from normal kidney tissue adjacent to a kidney cancer. Furthermore, there appears to be substantial expression in normal stomach, normal liver adjacent to a cancer, normal breast adjacent to a cancer and normal ovary adjacent to a cancer. Thus, the expression of this gene could be used to distinguish these normal tissues from their malignant counterparts. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be of use in the treatment of kidney, liver, breast or ovarian cancer.

#### **Panel 4D Summary: Ag2311**

NOV10 is modestly expressed (CT values = 30-33) in a wide variety of immune cell types and tissues. The highest expression of this gene is found in B cells stimulated with PWM and anti-CD40, where stimulation normally leads to the production of immunoglobulin (Ig) and Ig switching. High levels of expression of this transcript are also found in a pulmonary muco-epidermoid cell line (H292) treated with Th2 cytokines. These findings suggest that the NOV10 product may be important in the pathogenesis, and/or treatment of autoimmune

diseases such as lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, allergies which are associated with hyper IgE production, and lung inflammatory diseases such as asthma and emphysema. In addition, the high expression of this gene in the kidney suggests that the protein encoded by this transcript may be involved in normal tissue/cellular functions particularly in the kidney.

### NOV11a, NOV11b

Expression of NOV11a and NOV11b was assessed using the primer-probe set Ag3670, described in Table 72. Results of the RTQ-PCR runs are shown in Tables 73 and 74.

**Table 72. Probe Name Ag3670**

| Primers | Sequences                                          | Length | Start Position | SEQ ID NO: |
|---------|----------------------------------------------------|--------|----------------|------------|
| Forward | 5'-ACGAGGTCTTCATCAAGCTG-3'                         | 20     | 705            | 187        |
| Probe   | TET-5'-<br>CACCAACAAGTACAGCACCTTCTCCG-<br>3'-TAMRA | 26     | 751            | 188        |
| Reverse | 5'-CAGTCGGGGTAGATGATGAA-3'                         | 20     | 779            | 189        |

**Table 73. General\_screening\_panel\_v1.4**

| Tissue Name                      | Rel. Exp.(%)<br>Ag3670,<br>Run<br>216517130 | Rel. Exp.(%)<br>Ag3670,<br>Run<br>222735036 | Tissue Name                         | Rel. Exp.(%)<br>Ag3670,<br>Run<br>216517130 | Rel. Exp.(%)<br>Ag3670,<br>Run<br>222735036 |
|----------------------------------|---------------------------------------------|---------------------------------------------|-------------------------------------|---------------------------------------------|---------------------------------------------|
| Adipose                          | 0.2                                         | 0.4                                         | Renal ca. TK-10                     | 2.1                                         | 1.1                                         |
| Melanoma*<br>Hs688(A).T          | 0.0                                         | 0.0                                         | Bladder                             | 0.4                                         | 0.0                                         |
| Melanoma*<br>Hs688(B).T          | 0.0                                         | 0.0                                         | Gastric ca. (liver met.)<br>NCI-N87 | 0.8                                         | 0.4                                         |
| Melanoma* M14                    | 6.0                                         | 6.9                                         | Gastric ca. KATO III                | 0.4                                         | 0.4                                         |
| Melanoma*<br>LOXIMVI             | 2.6                                         | 1.8                                         | Colon ca. SW-948                    | 0.0                                         | 0.1                                         |
| Melanoma* SK-<br>MEL-5           | 5.9                                         | 8.2                                         | Colon ca. SW480                     | 2.6                                         | 3.5                                         |
| Squamous cell<br>carcinoma SCC-4 | 0.2                                         | 0.0                                         | Colon ca.* (SW480<br>met) SW620     | 1.3                                         | 0.8                                         |
| Testis Pool                      | 3.2                                         | 1.5                                         | Colon ca. HT29                      | 0.1                                         | 0.0                                         |
| Prostate ca.* (bone<br>met) PC-3 | 11.0                                        | 11.7                                        | Colon ca. HCT-116                   | 27.0                                        | 25.9                                        |
| Prostate Pool                    | 0.0                                         | 0.1                                         | Colon ca. CaCo-2                    | 11.7                                        | 13.3                                        |
| Placenta                         | 0.0                                         | 0.0                                         | Colon cancer tissue                 | 0.0                                         | 0.3                                         |

|                       |      |      |                                  |      |      |
|-----------------------|------|------|----------------------------------|------|------|
| Uterus Pool           | 0.1  | 0.1  | Colon ca. SW1116                 | 12.9 | 7.2  |
| Ovarian ca. OVCAR-3   | 2.3  | 1.2  | Colon ca. Colo-205               | 0.4  | 0.2  |
| Ovarian ca. SK-OV-3   | 1.9  | 3.2  | Colon ca. SW-48                  | 0.0  | 0.3  |
| Ovarian ca. OVCAR-4   | 1.5  | 1.5  | Colon Pool                       | 0.0  | 0.4  |
| Ovarian ca. OVCAR-5   | 6.3  | 6.0  | Small Intestine Pool             | 0.0  | 0.0  |
| Ovarian ca. IGROV-1   | 0.6  | 1.6  | Stomach Pool                     | 0.1  | 0.2  |
| Ovarian ca. OVCAR-8   | 6.9  | 7.9  | Bone Marrow Pool                 | 0.2  | 0.1  |
| Ovary                 | 0.0  | 0.0  | Fetal Heart                      | 0.0  | 0.0  |
| Breast ca. MCF-7      | 1.5  | 2.7  | Heart Pool                       | 0.0  | 0.0  |
| Breast ca. MDA-MB-231 | 0.8  | 0.6  | Lymph Node Pool                  | 0.2  | 0.2  |
| Breast ca. BT 549     | 6.8  | 11.6 | Fetal Skeletal Muscle            | 0.0  | 0.0  |
| Breast ca. T47D       | 21.8 | 16.8 | Skeletal Muscle Pool             | 0.0  | 0.0  |
| Breast ca. MDA-N      | 4.0  | 2.8  | Spleen Pool                      | 0.0  | 0.0  |
| Breast Pool           | 0.0  | 0.0  | Thymus Pool                      | 0.3  | 0.1  |
| Trachea               | 0.0  | 0.3  | CNS cancer (glio/astro) U87-MG   | 0.5  | 1.2  |
| Lung                  | 0.0  | 0.0  | CNS cancer (glio/astro) U-118-MG | 3.2  | 3.2  |
| Fetal Lung            | 0.0  | 0.0  | CNS cancer (neuro;met) SK-N-AS   | 6.4  | 10.0 |
| Lung ca. NCI-N417     | 5.7  | 4.5  | CNS cancer (astro) SF-539        | 2.6  | 0.8  |
| Lung ca. LX-1         | 0.2  | 0.5  | CNS cancer (astro) SNB-75        | 0.9  | 1.6  |
| Lung ca. NCI-H146     | 0.5  | 0.8  | CNS cancer (glio) SNB-19         | 0.3  | 0.8  |
| Lung ca. SHP-77       | 0.6  | 2.7  | CNS cancer (glio) SF-295         | 4.9  | 4.4  |
| Lung ca. A549         | 6.8  | 6.6  | Brain (Amygdala) Pool            | 0.0  | 0.3  |
| Lung ca. NCI-H526     | 1.6  | 2.5  | Brain (cerebellum)               | 0.0  | 0.3  |
| Lung ca. NCI-H23      | 16.4 | 12.7 | Brain (fetal)                    | 2.0  | 2.7  |
| Lung ca. NCI-H460     | 0.3  | 0.0  | Brain (Hippocampus) Pool         | 0.3  | 0.8  |
| Lung ca. HOP-62       | 0.6  | 0.8  | Cerebral Cortex Pool             | 0.0  | 0.2  |
| Lung ca. NCI-H522     | 48.6 | 46.0 | Brain (Substantia nigra) Pool    | 0.4  | 0.7  |
| Liver                 | 0.0  | 0.0  | Brain (Thalamus) Pool            | 0.4  | 0.9  |
| Fetal Liver           | 0.0  | 0.0  | Brain (whole)                    | 0.1  | 0.1  |

|                 |       |       |                          |     |     |
|-----------------|-------|-------|--------------------------|-----|-----|
| Liver ca. HepG2 | 0.1   | 0.3   | Spinal Cord Pool         | 0.2 | 0.3 |
| Kidney Pool     | 0.1   | 0.0   | Adrenal Gland            | 0.0 | 0.0 |
| Fetal Kidney    | 1.0   | 1.9   | Pituitary gland Pool     | 0.0 | 0.3 |
| Renal ca. 786-0 | 100.0 | 100.0 | Salivary Gland           | 0.0 | 0.2 |
| Renal ca. A498  | 10.2  | 21.3  | Thyroid (female)         | 1.2 | 0.3 |
| Renal ca. ACHN  | 0.5   | 0.2   | Pancreatic ca.<br>CAPAN2 | 0.9 | 0.2 |
| Renal ca. UO-31 | 1.5   | 1.8   | Pancreas Pool            | 0.0 | 0.2 |

Table 74. Panel 4.1D

| Tissue Name                       | Rel.<br>Exp.(%)<br>Ag3670,<br>Run<br>223785547 | Tissue Name                                    | Rel.<br>Exp.(%)<br>Ag3670,<br>Run<br>223785547 |
|-----------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|
| Secondary Th1 act                 | 0.0                                            | HUVEC IL-1beta                                 | 27.5                                           |
| Secondary Th2 act                 | 0.0                                            | HUVEC IFN gamma                                | 29.9                                           |
| Secondary Tr1 act                 | 0.0                                            | HUVEC TNF alpha + IFN gamma                    | 19.5                                           |
| Secondary Th1 rest                | 0.0                                            | HUVEC TNF alpha + IL4                          | 0.0                                            |
| Secondary Th2 rest                | 0.0                                            | HUVEC IL-11                                    | 0.0                                            |
| Secondary Tr1 rest                | 0.0                                            | Lung Microvascular EC none                     | 0.0                                            |
| Primary Th1 act                   | 0.0                                            | Lung Microvascular EC<br>TNFalpha + IL-1beta   | 11.6                                           |
| Primary Th2 act                   | 0.0                                            | Microvascular Dermal EC none                   | 0.0                                            |
| Primary Tr1 act                   | 0.0                                            | Microvascular Dermal EC<br>TNFalpha + IL-1beta | 71.7                                           |
| Primary Th1 rest                  | 0.0                                            | Bronchial epithelium TNFalpha +<br>IL1beta     | 53.2                                           |
| Primary Th2 rest                  | 0.0                                            | Small airway epithelium none                   | 0.0                                            |
| Primary Tr1 rest                  | 0.0                                            | Small airway epithelium<br>TNFalpha + IL-1beta | 0.0                                            |
| CD45RA CD4 lymphocyte act         | 0.0                                            | Coronary artery SMC rest                       | 0.0                                            |
| CD45RO CD4 lymphocyte act         | 0.0                                            | Coronary artery SMC TNFalpha +<br>IL-1beta     | 0.0                                            |
| CD8 lymphocyte act                | 15.1                                           | Astrocytes rest                                | 0.0                                            |
| Secondary CD8 lymphocyte rest     | 0.0                                            | Astrocytes TNFalpha + IL-1beta                 | 0.0                                            |
| Secondary CD8 lymphocyte act      | 39.5                                           | KU-812 (Basophil) rest                         | 0.0                                            |
| CD4 lymphocyte none               | 0.0                                            | KU-812 (Basophil)<br>PMA/ionomycin             | 0.0                                            |
| 2ry Th1/Th2/Tr1_anti-CD95<br>CH11 | 0.0                                            | CCD1106 (Keratinocytes) none                   | 0.0                                            |
| LAK cells rest                    | 0.0                                            | CCD1106 (Keratinocytes)<br>TNFalpha + IL-1beta | 38.2                                           |

|                               |       |                                       |      |
|-------------------------------|-------|---------------------------------------|------|
| LAK cells IL-2                | 0.0   | Liver cirrhosis                       | 0.0  |
| LAK cells IL-2+IL-12          | 0.0   | NCI-H292 none                         | 0.0  |
| LAK cells IL-2+IFN gamma      | 0.0   | NCI-H292 IL-4                         | 34.9 |
| LAK cells IL-2+ IL-18         | 0.0   | NCI-H292 IL-9                         | 24.8 |
| LAK cells PMA/ionomycin       | 0.0   | NCI-H292 IL-13                        | 21.5 |
| NK Cells IL-2 rest            | 0.0   | NCI-H292 IFN gamma                    | 33.9 |
| Two Way MLR 3 day             | 0.0   | HPAEC none                            | 0.0  |
| Two Way MLR 5 day             | 0.0   | HPAEC TNF alpha + IL-1 beta           | 0.0  |
| Two Way MLR 7 day             | 0.0   | Lung fibroblast none                  | 12.8 |
| PBMC rest                     | 0.0   | Lung fibroblast TNF alpha + IL-1 beta | 0.0  |
| PBMC PWM                      | 0.0   | Lung fibroblast IL-4                  | 29.3 |
| PBMC PHA-L                    | 0.0   | Lung fibroblast IL-9                  | 46.7 |
| Ramos (B cell) none           | 0.0   | Lung fibroblast IL-13                 | 0.0  |
| Ramos (B cell) ionomycin      | 0.0   | Lung fibroblast IFN gamma             | 0.0  |
| B lymphocytes PWM             | 0.0   | Dermal fibroblast CCD1070 rest        | 24.5 |
| B lymphocytes CD40L and IL-4  | 0.0   | Dermal fibroblast CCD1070 TNF alpha   | 22.8 |
| EOL-1 dbcAMP                  | 100.0 | Dermal fibroblast CCD1070 IL-1 beta   | 0.0  |
| EOL-1 dbcAMP<br>PMA/ionomycin | 45.1  | Dermal fibroblast IFN gamma           | 0.0  |
| Dendritic cells none          | 0.0   | Dermal fibroblast IL-4                | 0.0  |
| Dendritic cells LPS           | 0.0   | Dermal Fibroblasts rest               | 0.0  |
| Dendritic cells anti-CD40     | 0.0   | Neutrophils TNFa+LPS                  | 0.0  |
| Monocytes rest                | 0.0   | Neutrophils rest                      | 0.0  |
| Monocytes LPS                 | 0.0   | Colon                                 | 0.0  |
| Macrophages rest              | 0.0   | Lung                                  | 0.0  |
| Macrophages LPS               | 0.0   | Thymus                                | 0.0  |
| HUVEC none                    | 25.9  | Kidney                                | 59.5 |
| HUVEC starved                 | 0.0   |                                       |      |

#### General\_screening\_panel\_v1.4 Summary: Ag3670

Two experiments with the same probe and primer sets show results that are in excellent agreement, with highest expression in a renal cancer cell line. In general, the expression of this gene appears to be largely associated with samples derived from cancer cell lines rather than normal tissues. Of note is the substantial expression associated with kidney cancer cell lines as well as in colon cancer and lung cancer cell lines. Thus, the expression of this gene could be used to distinguish these cell lines from other cell lines. Moreover, therapeutic modulation of

this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be of use in the treatment of kidney, colon or lung cancer.

This gene is a C1q-related factor variant, and is expressed in at least the fetal brain, hippocampus, substantia nigra and thalamus. Various members of the complement cascade have been implicated in neuroinflammation and the pathology of Alzheimer's disease. Recent case controlled studies also suggest that the use of anti-inflammatory agents decreases the risk of Alzheimer's disease. Therefore, this gene is an excellent drug target for the disruption of neuroinflammation and the treatment of Alzheimer's disease, Huntington's disease, and stroke.

#### References:

Lue LF, Rydel R, Brigham EF, Yang LB, Hampel H, Murphy GM Jr, Brachova L, Yan SD, Walker DG, Shen Y, Rogers J. Inflammatory repertoire of Alzheimer's disease and nondemented elderly microglia in vitro. *Glia* 2001 Jul;35(1):72-9

In this study complement activation and biosynthesis have been analysed in the brains of Huntington's disease (HD) (n = 9) and normal (n = 3) individuals. In HD striatum, neurons, myelin and astrocytes were strongly stained with antibodies to C1q, C4, C3, iC3b-neoepitope and C9-neoepitope. In contrast, no staining for complement components was found in the normal striatum. Marked astrogliosis and microgliosis were observed in all HD caudate and the internal capsule samples but not in normal brain. RT-PCR analysis and in-situ hybridisation were carried out to determine whether complement was synthesised locally by activated glial cells. By RT-PCR, we found that complement activators of the classical pathway C1q C chain, C1r, C4, C3, as well as the complement regulators, C1 inhibitor, clusterin, MCP, DAF, CD59, were all expressed constitutively and at much higher level in HD brains compared to normal brain. Complement anaphylatoxin receptor mRNAs (C5a receptor and C3a receptor) were strongly expressed in HD caudate. In general, we found that the level of complement mRNA in normal control brains was from 2 to 5 fold lower compared to HD striatum. Using in-situ hybridisation, we confirmed that C3 mRNA and C9 mRNA were expressed by reactive microglia in HD internal capsule. We propose that complement produced locally by reactive microglia is activated on the membranes of neurons, contributing to neuronal necrosis but also to proinflammatory activities. Complement opsonins (iC3b) and anaphylatoxins (C3a, C5a) may be involved in the recruitment and stimulation of glial cells and phagocytes bearing specific complement receptors.

#### Panel 4.1D Summary: Ag3670



The NOV11 transcript, which encodes a protein with homology to a C1q related factor, is expressed at a low level in eosinophils, microvascular dermal endothelial cells and bronchial epithelium. The inflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  appear to up-regulate expression of this transcript in the endothelial cells and bronchial epithelium. This suggests that expression of this transcript is regulated by inflammatory conditions such as those found in lung inflammatory disease including pneumonia and bronchitis as well as skin infection or wounds. Expression of this transcript is also up regulated in lung fibroblasts by the Th2 cytokines IL9 or IL4, conditions found in asthma and COPD. The expression of this transcript in eosinophils, cells that are frequently associated with asthma, ulcerative colitis or other Th2 mediated diseases strongly suggest that modulation of the expression of this transcript will be beneficial in the treatment of atopic lung and skin diseases. Since the C1q factor is usually involved in the activation of complement and innate immunity, modulation of the expression of this transcript could modulate excessive inflammatory processes leading to these diseases.

**Panel 5D Summary:** Expression is low/undetectable for all samples in this panel (CT>35). (Data not shown).

## NOV12

Expression of NOV12 was assessed using the primer-probe sets Ag1586 and Ag2011, described in Tables 75 and 76. Results of the RTQ-PCR runs are shown in Tables 77, 78, 79 and 80.

**Table 75. Probe Name Ag1586**

| Primers | Sequences                                         | Length | Start Position | SEQ ID NO: |
|---------|---------------------------------------------------|--------|----------------|------------|
| Forward | 5'-ACCAGGATGAGTTGTGTCATC-3'                       | 22     | 735            | 190        |
| Probe   | TET-5'-<br>CTCAAGATCCCTTCGGACACGCTGT-<br>3'-TAMRA | 25     | 761            | 191        |
| Reverse | 5'-TGC GGAAGCTGTACACATAGTA-3'                     | 22     | 809            | 192        |

**Table 76. Probe Name Ag2011**

| Primers | Sequences                                         | Length | Start Position | SEQ ID NO: |
|---------|---------------------------------------------------|--------|----------------|------------|
| Forward | 5'-ACCAGGATGAGTTGTGTCATC-3'                       | 22     | 735            | 193        |
| Probe   | TET-5'-<br>CTCAAGATCCCTTCGGACACGCTGT-3'-<br>TAMRA | 25     | 761            | 194        |
| Reverse | 5'-TGC GGAAGCTGTACACATAGTA-3'                     | 22     | 809            | 195        |

Table 77. Panel 1.3D

| Tissue Name              | Rel.<br>Exp.(%)<br>Ag1586,<br>Run<br>146473155 | Rel.<br>Exp.(%)<br>Ag2011,<br>Run<br>147816085 | Tissue Name                         | Rel.<br>Exp.(%)<br>Ag1586,<br>Run<br>146473155 | Rel.<br>Exp.(%)<br>Ag2011,<br>Run<br>147816085 |
|--------------------------|------------------------------------------------|------------------------------------------------|-------------------------------------|------------------------------------------------|------------------------------------------------|
| Liver adenocarcinoma     | 29.9                                           | 37.6                                           | Kidney (fetal)                      | 3.8                                            | 3.7                                            |
| Pancreas                 | 1.7                                            | 0.7                                            | Renal ca. 786-0                     | 6.1                                            | 11.7                                           |
| Pancreatic ca. CAPAN 2   | 6.3                                            | 9.6                                            | Renal ca. A498                      | 25.0                                           | 25.9                                           |
| Adrenal gland            | 2.6                                            | 2.5                                            | Renal ca. RXF 393                   | 4.5                                            | 5.0                                            |
| Thyroid                  | 2.5                                            | 1.8                                            | Renal ca. ACHN                      | 8.8                                            | 11.3                                           |
| Salivary gland           | 1.9                                            | 2.2                                            | Renal ca. UO-31                     | 15.0                                           | 15.0                                           |
| Pituitary gland          | 0.9                                            | 1.5                                            | Renal ca. TK-10                     | 4.4                                            | 4.6                                            |
| Brain (fetal)            | 12.2                                           | 13.1                                           | Liver                               | 0.2                                            | 0.1                                            |
| Brain (whole)            | 9.7                                            | 10.7                                           | Liver (fetal)                       | 0.7                                            | 0.8                                            |
| Brain (amygdala)         | 9.5                                            | 9.9                                            | Liver ca.<br>(hepatoblast)<br>HepG2 | 16.8                                           | 12.8                                           |
| Brain (cerebellum)       | 3.3                                            | 2.3                                            | Lung                                | 5.0                                            | 5.1                                            |
| Brain (hippocampus)      | 24.7                                           | 21.0                                           | Lung (fetal)                        | 7.4                                            | 8.1                                            |
| Brain (substantia nigra) | 0.9                                            | 1.3                                            | Lung ca. (small<br>cell) LX-1       | 16.8                                           | 12.1                                           |
| Brain (thalamus)         | 4.7                                            | 3.7                                            | Lung ca. (small<br>cell) NCI-H69    | 18.4                                           | 23.7                                           |
| Cerebral Cortex          | 75.8                                           | 71.2                                           | Lung ca. (s.cell<br>var.) SHP-77    | 8.5                                            | 7.2                                            |
| Spinal cord              | 2.0                                            | 2.4                                            | Lung ca. (large<br>cell) NCI-H460   | 10.7                                           | 10.1                                           |
| glio/astro U87-MG        | 15.3                                           | 17.9                                           | Lung ca. (non-sm.<br>cell) A549     | 3.2                                            | 4.1                                            |
| glio/astro U-118-MG      | 38.2                                           | 41.2                                           | Lung ca. (non-<br>s.cell) NCI-H23   | 23.2                                           | 24.7                                           |
| astrocytoma SW1783       | 8.3                                            | 10.4                                           | Lung ca. (non-<br>s.cell) HOP-62    | 18.9                                           | 15.7                                           |
| neuro*; met SK-N-AS      | 23.5                                           | 24.3                                           | Lung ca. (non-s.cl)<br>NCI-H522     | 5.6                                            | 7.5                                            |
| astrocytoma SF-539       | 19.6                                           | 38.4                                           | Lung ca. (squam.)<br>SW 900         | 13.0                                           | 13.1                                           |
| astrocytoma SNB-75       | 44.4                                           | 45.1                                           | Lung ca. (squam.)<br>NCI-H596       | 6.5                                            | 5.7                                            |
| glioma SNB-19            | 26.2                                           | 12.2                                           | Mammary gland                       | 11.5                                           | 9.3                                            |
| glioma U251              | 16.4                                           | 16.2                                           | Breast ca.* (pl.ef)<br>MCF-7        | 14.1                                           | 14.4                                           |
| glioma SF-295            | 26.4                                           | 36.9                                           | Breast ca.* (pl.ef)<br>MDA-MB-231   | 82.9                                           | 87.1                                           |

|                                    |      |      |                                  |       |       |
|------------------------------------|------|------|----------------------------------|-------|-------|
| Heart (Fetal)                      | 80.7 | 95.3 | Breast ca.* (pl. ef)<br>T47D     | 6.1   | 4.6   |
| Heart                              | 2.8  | 1.9  | Breast ca. BT-549                | 13.6  | 11.2  |
| Skeletal muscle (Fetal)            | 85.3 | 87.7 | Breast ca. MDA-N                 | 28.1  | 31.6  |
| Skeletal muscle                    | 2.1  | 2.4  | Ovary                            | 20.9  | 19.5  |
| Bone marrow                        | 0.6  | 0.3  | Ovarian ca.<br>OVCAR-3           | 33.0  | 40.1  |
| Thymus                             | 2.6  | 2.3  | Ovarian ca.<br>OVCAR-4           | 5.5   | 5.4   |
| Spleen                             | 2.9  | 2.6  | Ovarian ca.<br>OVCAR-5           | 10.9  | 13.1  |
| Lymph node                         | 5.1  | 5.2  | Ovarian ca.<br>OVCAR-8           | 17.4  | 18.3  |
| Colorectal                         | 5.2  | 3.9  | Ovarian ca.<br>IGROV-1           | 4.5   | 5.3   |
| Stomach                            | 3.7  | 5.6  | Ovarian ca.<br>(ascites) SK-OV-3 | 25.7  | 22.4  |
| Small intestine                    | 1.6  | 1.3  | Uterus                           | 2.7   | 2.4   |
| Colon ca. SW480                    | 45.4 | 55.5 | Placenta                         | 6.7   | 10.2  |
| Colon ca.* SW620<br>(SW480 met)    | 11.3 | 11.1 | Prostate                         | 0.4   | 1.4   |
| Colon ca. HT29                     | 13.3 | 13.3 | Prostate ca.* (bone<br>met) PC-3 | 8.4   | 11.3  |
| Colon ca. HCT-116                  | 10.5 | 10.5 | Testis                           | 8.1   | 8.5   |
| Colon ca. CaCo-2                   | 24.0 | 23.0 | Melanoma<br>Hs688(A).T           | 59.0  | 86.5  |
| CC Well to Mod Diff<br>(ODO3866)   | 19.1 | 16.6 | Melanoma* (met)<br>Hs688(B).T    | 100.0 | 100.0 |
| Colon ca. HCC-2998                 | 25.7 | 20.3 | Melanoma UACC-<br>62             | 17.6  | 19.5  |
| Gastric ca. (liver met)<br>NCI-N87 | 59.9 | 62.9 | Melanoma M14                     | 16.3  | 21.9  |
| Bladder                            | 1.8  | 4.6  | Melanoma LOX<br>IMVI             | 3.6   | 5.8   |
| Trachea                            | 6.9  | 5.6  | Melanoma* (met)<br>SK-MEL-5      | 12.9  | 22.1  |
| Kidney                             | 0.8  | 0.7  | Adipose                          | 5.6   | 4.5   |

Table 78. Panel 2.2

| Tissue Name            | Rel.<br>Exp.(%)<br>Ag2011,<br>Run<br>174154748 | Tissue Name             | Rel.<br>Exp.(%)<br>Ag2011,<br>Run<br>174154748 |
|------------------------|------------------------------------------------|-------------------------|------------------------------------------------|
| Normal Colon           | 24.7                                           | Kidney Margin (OD04348) | 68.3                                           |
| Colon cancer (OD06064) | 48.6                                           | Kidney malignant cancer | 25.0                                           |

|                                             |       |                                          |      |
|---------------------------------------------|-------|------------------------------------------|------|
|                                             |       | (OD06204B)                               |      |
| Colon Margin (OD06064)                      | 4.9   | Kidney normal adjacent tissue (OD06204E) | 7.4  |
| Colon cancer (OD06159)                      | 9.3   | Kidney Cancer (OD04450-01)               | 34.4 |
| Colon Margin (OD06159)                      | 19.5  | Kidney Margin (OD04450-03)               | 18.4 |
| Colon cancer (OD06297-04)                   | 11.7  | Kidney Cancer 8120613                    | 9.7  |
| Colon Margin (OD06297-015)                  | 12.5  | Kidney Margin 8120614                    | 18.8 |
| CC Gr.2 ascend colon (ODO3921)              | 17.3  | Kidney Cancer 9010320                    | 16.2 |
| CC Margin (ODO3921)                         | 14.2  | Kidney Margin 9010321                    | 13.8 |
| Colon cancer metastasis (OD06104)           | 8.6   | Kidney Cancer 8120607                    | 37.1 |
| Lung Margin (OD06104)                       | 8.3   | Kidney Margin 8120608                    | 7.0  |
| Colon mets to lung (OD04451-01)             | 23.0  | Normal Uterus                            | 21.9 |
| Lung Margin (OD04451-02)                    | 32.8  | Uterine Cancer 064011                    | 13.7 |
| Normal Prostate                             | 4.8   | Normal Thyroid                           | 2.4  |
| Prostate Cancer (OD04410)                   | 4.9   | Thyroid Cancer                           | 8.1  |
| Prostate Margin (OD04410)                   | 8.8   | Thyroid Cancer A302152                   | 35.4 |
| Normal Ovary                                | 32.3  | Thyroid Margin A302153                   | 8.7  |
| Ovarian cancer (OD06283-03)                 | 32.1  | Normal Breast                            | 29.7 |
| Ovarian Margin (OD06283-07)                 | 13.8  | Breast Cancer                            | 11.9 |
| Ovarian Cancer                              | 19.9  | Breast Cancer                            | 47.6 |
| Ovarian cancer (OD06145)                    | 9.2   | Breast Cancer (OD04590-01)               | 25.5 |
| Ovarian Margin (OD06145)                    | 8.6   | Breast Cancer Mets (OD04590-03)          | 38.4 |
| Ovarian cancer (OD06455-03)                 | 13.0  | Breast Cancer Metastasis                 | 30.1 |
| Ovarian Margin (OD06455-07)                 | 2.1   | Breast Cancer                            | 41.5 |
| Normal Lung                                 | 27.2  | Breast Cancer 9100266                    | 9.2  |
| Invasive poor diff. lung adeno (ODO4945-01) | 28.5  | Breast Margin 9100265                    | 18.2 |
| Lung Margin (ODO4945-03)                    | 15.0  | Breast Cancer A209073                    | 14.9 |
| Lung Malignant Cancer (OD03126)             | 30.4  | Breast Margin A2090734                   | 37.6 |
| Lung Margin (OD03126)                       | 15.9  | Breast cancer (OD06083)                  | 55.9 |
| Lung Cancer (OD05014A)                      | 39.5  | Breast cancer node metastasis (OD06083)  | 48.6 |
| Lung Margin (OD05014B)                      | 22.1  | Normal Liver                             | 10.4 |
| Lung cancer (OD06081)                       | 23.7  | Liver Cancer 1026                        | 9.1  |
| Lung Margin (OD06081)                       | 16.8  | Liver Cancer 1025                        | 20.7 |
| Lung Cancer (OD04237-01)                    | 9.0   | Liver Cancer 6004-T                      | 12.2 |
| Lung Margin (OD04237-02)                    | 41.5  | Liver Tissue 6004-N                      | 8.0  |
| Ocular Mel Met to Liver (ODO4310)           | 100.0 | Liver Cancer 6005-T                      | 36.6 |

|                                       |      |                        |      |
|---------------------------------------|------|------------------------|------|
| Liver Margin (ODO4310)                | 4.2  | Liver Tissue 6005-N    | 25.0 |
| Melanoma Metastasis                   | 47.0 | Liver Cancer           | 4.5  |
| Lung Margin (OD04321)                 | 28.1 | Normal Bladder         | 18.7 |
| Normal Kidney                         | 12.3 | Bladder Cancer         | 17.2 |
| Kidney Ca, Nuclear grade 2 (OD04338)  | 18.3 | Bladder Cancer         | 72.7 |
| Kidney Margin (OD04338)               | 18.0 | Normal Stomach         | 33.4 |
| Kidney Ca Nuclear grade 1/2 (OD04339) | 83.5 | Gastric Cancer 9060397 | 9.6  |
| Kidney Margin (OD04339)               | 10.4 | Stomach Margin 9060396 | 10.4 |
| Kidney Ca, Clear cell type (OD04340)  | 22.2 | Gastric Cancer 9060395 | 7.6  |
| Kidney Margin (OD04340)               | 12.7 | Stomach Margin 9060394 | 19.6 |
| Kidney Ca, Nuclear grade 3 (OD04348)  | 15.7 | Gastric Cancer 064005  | 17.4 |

Table 79. Panel 2D

| Tissue Name                                | Rel.<br>Exp.(%)<br>Ag1586,<br>Run<br>162624476 | Tissue Name                     | Rel.<br>Exp.(%)<br>Ag1586,<br>Run<br>162624476 |
|--------------------------------------------|------------------------------------------------|---------------------------------|------------------------------------------------|
| Normal Colon                               | 34.9                                           | Kidney Margin 8120608           | 14.2                                           |
| CC Well to Mod Diff (ODO3866)              | 28.3                                           | Kidney Cancer 8120613           | 30.4                                           |
| CC Margin (ODO3866)                        | 9.2                                            | Kidney Margin 8120614           | 17.7                                           |
| CC Gr.2 rectosigmoid (ODO3868)             | 25.9                                           | Kidney Cancer 9010320           | 57.0                                           |
| CC Margin (ODO3868)                        | 4.7                                            | Kidney Margin 9010321           | 40.9                                           |
| CC Mod Diff (ODO3920)                      | 55.5                                           | Normal Uterus                   | 10.4                                           |
| CC Margin (ODO3920)                        | 14.2                                           | Uterine Cancer 064011           | 28.9                                           |
| CC Gr.2 ascend colon (ODO3921)             | 62.9                                           | Normal Thyroid                  | 8.4                                            |
| CC Margin (ODO3921)                        | 12.1                                           | Thyroid Cancer                  | 16.7                                           |
| CC from Partial Hepatectomy (ODO4309) Mets | 41.5                                           | Thyroid Cancer A302152          | 24.7                                           |
| Liver Margin (ODO4309)                     | 13.6                                           | Thyroid Margin A302153          | 17.7                                           |
| Colon mets to lung (OD04451-01)            | 18.0                                           | Normal Breast                   | 60.3                                           |
| Lung Margin (OD04451-02)                   | 25.5                                           | Breast Cancer                   | 24.1                                           |
| Normal Prostate 6546-1                     | 17.0                                           | Breast Cancer (OD04590-01)      | 47.0                                           |
| Prostate Cancer (OD04410)                  | 33.7                                           | Breast Cancer Mets (OD04590-03) | 72.7                                           |
| Prostate Margin (OD04410)                  | 28.9                                           | Breast Cancer Metastasis        | 37.4                                           |
| Prostate Cancer (OD04720-01)               | 33.7                                           | Breast Cancer                   | 36.9                                           |

|                                       |       |                                      |      |
|---------------------------------------|-------|--------------------------------------|------|
| Prostate Margin (OD04720-02)          | 45.7  | Breast Cancer                        | 65.1 |
| Normal Lung                           | 80.7  | Breast Cancer 9100266                | 39.8 |
| Lung Met to Muscle (ODO4286)          | 100.0 | Breast Margin 9100265                | 31.2 |
| Muscle Margin (ODO4286)               | 21.5  | Breast Cancer A209073                | 49.0 |
| Lung Malignant Cancer (OD03126)       | 57.8  | Breast Margin A2090734               | 44.8 |
| Lung Margin (OD03126)                 | 61.6  | Normal Liver                         | 4.5  |
| Lung Cancer (OD04404)                 | 70.2  | Liver Cancer                         | 2.6  |
| Lung Margin (OD04404)                 | 34.2  | Liver Cancer 1025                    | 4.7  |
| Lung Cancer (OD04565)                 | 87.7  | Liver Cancer 1026                    | 18.3 |
| Lung Margin (OD04565)                 | 23.8  | Liver Cancer 6004-T                  | 7.6  |
| Lung Cancer (OD04237-01)              | 41.5  | Liver Tissue 6004-N                  | 12.0 |
| Lung Margin (OD04237-02)              | 34.2  | Liver Cancer 6005-T                  | 12.1 |
| Ocular Mel Met to Liver (ODO4310)     | 97.3  | Liver Tissue 6005-N                  | 5.7  |
| Liver Margin (ODO4310)                | 5.0   | Normal Bladder                       | 38.2 |
| Melanoma Metastasis                   | 87.7  | Bladder Cancer                       | 21.3 |
| Lung Margin (OD04321)                 | 56.3  | Bladder Cancer                       | 46.0 |
| Normal Kidney                         | 30.1  | Bladder Cancer (OD04718-01)          | 96.6 |
| Kidney Ca, Nuclear grade 2 (OD04338)  | 46.7  | Bladder Normal Adjacent (OD04718-03) | 29.5 |
| Kidney Margin (OD04338)               | 14.8  | Normal Ovary                         | 21.5 |
| Kidney Ca Nuclear grade 1/2 (OD04339) | 52.1  | Ovarian Cancer                       | 73.7 |
| Kidney Margin (OD04339)               | 20.3  | Ovarian Cancer (OD04768-07)          | 48.3 |
| Kidney Ca, Clear cell type (OD04340)  | 49.0  | Ovary Margin (OD04768-08)            | 18.8 |
| Kidney Margin (OD04340)               | 23.2  | Normal Stomach                       | 13.9 |
| Kidney Ca, Nuclear grade 3 (OD04348)  | 42.6  | Gastric Cancer 9060358               | 6.7  |
| Kidney Margin (OD04348)               | 28.9  | Stomach Margin 9060359               | 13.2 |
| Kidney Cancer (OD04622-01)            | 50.7  | Gastric Cancer 9060395               | 28.3 |
| Kidney Margin (OD04622-03)            | 8.6   | Stomach Margin 9060394               | 18.0 |
| Kidney Cancer (OD04450-01)            | 21.8  | Gastric Cancer 9060397               | 45.4 |
| Kidney Margin (OD04450-03)            | 18.2  | Stomach Margin 9060396               | 10.4 |
| Kidney Cancer 8120607                 | 25.0  | Gastric Cancer 064005                | 48.3 |

Table 80. Panel 4D

| Tissue Name       | Rel. Exp.(%)<br>Ag2011, Run<br>160997385 | Tissue Name           | Rel. Exp.(%)<br>Ag2011, Run<br>160997385 |
|-------------------|------------------------------------------|-----------------------|------------------------------------------|
| Secondary Th1 act | 4.7                                      | HUVEC IL-1beta        | 2.0                                      |
| Secondary Th2 act | 6.4                                      | HUVEC IFN gamma       | 4.0                                      |
| Secondary Tr1 act | 8.6                                      | HUVEC TNF alpha + IFN | 5.0                                      |

|                                   |      |                                                |       |
|-----------------------------------|------|------------------------------------------------|-------|
|                                   |      | gamma                                          |       |
| Secondary Th1 rest                | 0.6  | HUVEC TNF alpha + IL4                          | 8.4   |
| Secondary Th2 rest                | 1.7  | HUVEC IL-11                                    | 3.5   |
| Secondary Tr1 rest                | 1.7  | Lung Microvascular EC none                     | 13.0  |
| Primary Th1 act                   | 14.0 | Lung Microvascular EC<br>TNFalpha + IL-1beta   | 15.3  |
| Primary Th2 act                   | 7.7  | Microvascular Dermal EC<br>none                | 23.2  |
| Primary Tr1 act                   | 12.9 | Microvascular Dermal EC<br>TNFalpha + IL-1beta | 17.3  |
| Primary Th1 rest                  | 3.3  | Bronchial epithelium<br>TNFalpha + IL1beta     | 4.5   |
| Primary Th2 rest                  | 2.3  | Small airway epithelium<br>none                | 16.0  |
| Primary Tr1 rest                  | 2.0  | Small airway epithelium<br>TNFalpha + IL-1beta | 100.0 |
| CD45RA CD4 lymphocyte<br>act      | 6.5  | Coronary artery SMC rest                       | 15.7  |
| CD45RO CD4 lymphocyte<br>act      | 5.3  | Coronary artery SMC<br>TNFalpha + IL-1beta     | 11.1  |
| CD8 lymphocyte act                | 3.3  | Astrocytes rest                                | 25.3  |
| Secondary CD8 lymphocyte<br>rest  | 7.2  | Astrocytes TNFalpha + IL-<br>1beta             | 21.6  |
| Secondary CD8 lymphocyte<br>act   | 3.0  | KU-812 (Basophil) rest                         | 8.4   |
| CD4 lymphocyte none               | 1.6  | KU-812 (Basophil)<br>PMA/ionomycin             | 39.5  |
| 2ry Th1/Th2/Tr1_anti-CD95<br>CH11 | 0.3  | CCD1106 (Keratinocytes)<br>none                | 35.1  |
| LAK cells rest                    | 19.1 | CCD1106 (Keratinocytes)<br>TNFalpha + IL-1beta | 5.9   |
| LAK cells IL-2                    | 3.1  | Liver cirrhosis                                | 0.9   |
| LAK cells IL-2+IL-12              | 6.5  | Lupus kidney                                   | 1.3   |
| LAK cells IL-2+IFN gamma          | 9.8  | NCI-H292 none                                  | 42.3  |
| LAK cells IL-2+ IL-18             | 5.9  | NCI-H292 IL-4                                  | 90.1  |
| LAK cells PMA/ionomycin           | 8.7  | NCI-H292 IL-9                                  | 58.2  |
| NK Cells IL-2 rest                | 1.7  | NCI-H292 IL-13                                 | 33.9  |
| Two Way MLR 3 day                 | 9.3  | NCI-H292 IFN gamma                             | 30.4  |
| Two Way MLR 5 day                 | 7.4  | HPAEC none                                     | 5.8   |
| Two Way MLR 7 day                 | 2.0  | HPAEC TNF alpha + IL-1<br>beta                 | 12.9  |
| PBMC rest                         | 1.7  | Lung fibroblast none                           | 23.8  |
| PBMC PWM                          | 12.5 | Lung fibroblast TNF alpha +<br>IL-1 beta       | 10.7  |
| PBMC PHA-L                        | 5.4  | Lung fibroblast IL-4                           | 59.0  |

|                              |      |                                     |      |
|------------------------------|------|-------------------------------------|------|
| Ramos (B cell) none          | 0.5  | Lung fibroblast IL-9                | 40.6 |
| Ramos (B cell) ionomycin     | 0.9  | Lung fibroblast IL-13               | 31.0 |
| B lymphocytes PWM            | 15.6 | Lung fibroblast IFN gamma           | 65.5 |
| B lymphocytes CD40L and IL-4 | 5.8  | Dermal fibroblast CCD1070 rest      | 37.4 |
| EOL-1 dbcAMP                 | 3.5  | Dermal fibroblast CCD1070 TNF alpha | 50.0 |
| EOL-1 dbcAMP PMA/ionomycin   | 60.3 | Dermal fibroblast CCD1070 IL-1 beta | 19.6 |
| Dendritic cells none         | 17.6 | Dermal fibroblast IFN gamma         | 15.0 |
| Dendritic cells LPS          | 32.5 | Dermal fibroblast IL-4              | 43.8 |
| Dendritic cells anti-CD40    | 21.0 | IBD Colitis 2                       | 0.3  |
| Monocytes rest               | 0.1  | IBD Crohn's                         | 0.8  |
| Monocytes LPS                | 8.4  | Colon                               | 5.3  |
| Macrophages rest             | 34.2 | Lung                                | 15.0 |
| Macrophages LPS              | 11.3 | Thymus                              | 5.8  |
| HUVEC none                   | 6.5  | Kidney                              | 11.4 |
| HUVEC starved                | 9.3  |                                     |      |

### Panel 1.3D Summary: Ag1586/Ag2011

Two experiments with the same probe and primer set produce results that are in excellent agreement. NOV12 appears to be expressed largely in cancer cell lines, with highest expression in a melanoma cell line (CTs=26-28). Of note is the expression associated with colon cancer cell lines as well as melanoma cell lines. Thus, the expression of this gene could be used to distinguish these samples from other samples on the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be of use in the treatment of colon cancer or melanoma.

This gene is modestly expressed in a variety of metabolic tissues including pancreas, adrenal, thyroid, pituitary, fetal liver, and adipose. Thus, this gene product may be an antibody target for the treatment of metabolic disease, including obesity and diabetes, in any or all of these tissues. In addition, NOV12 is differentially expressed in fetal (CT values = 26-28) versus adult heart (CT values = 31-33), and in fetal (CT values = 26-28) versus adult skeletal muscle (CT values = 32-33), and may be used to differentiate between the adult and fetal sources of these tissues. Furthermore, the higher levels of expression in the fetal tissues suggest that the SC132340676\_A gene product may be involved in the development of heart and skeletal muscle tissue. Thus, therapeutic modulation of the expression or function of the



protein encoded by the SC132340676\_A gene may be beneficial in the treatment of diseases that result in weak or dystrophic heart or skeletal muscle tissue, including ardiomyopathy, atherosclerosis, hypertension, congenital heart defects, aortic stenosis, atrial septal defect (ASD), atrioventricular (A-V) canal defect, ductus arteriosus , pulmonary stenosis, subaortic stenosis, ventricular septal defect (VSD), valve diseases, muscular dystrophy, Lesch-Nyhan syndrome, and myasthenia gravis.

This gene represents a novel protein with homology to a plexin that is expressed at moderate to high levels in all brain regions examined. Plexins act as receptors for semaphorins in the CNS. The interactions of the semaphorins and their receptors are critical for axon guidance. Therefore, this gene product may be useful as a drug target in clinical conditions where axonal growth and/or compensatory synaptogenesis are desireable (spinal cord or head trauma, stroke, or neurodegenerative diseases such as Alzheimer's, Parkinson's, or Huntington's disease).

#### References:

1. Pasterkamp RJ, Ruitenberg MJ, Verhaagen J. Semaphorins and their receptors in olfactory axon guidance. *Cell Mol Biol (Noisy-le-grand)* 1999 Sep;45(6):763-79

The mammalian olfactory system is capable of discriminating among a large variety of odor molecules and is therefore essential for the identification of food, enemies and mating partners. The assembly and maintenance of olfactory connectivity have been shown to depend on the combinatorial actions of a variety of molecular signals, including extracellular matrix, cell adhesion and odorant receptor molecules. Recent studies have identified semaphorins and their receptors as putative molecular cues involved in olfactory pathfinding, plasticity and regeneration. The semaphorins comprise a large family of secreted and transmembrane axon guidance proteins, being either repulsive or attractive in nature. Neuropilins were shown to serve as receptors for secreted class 3 semaphorins, whereas members of the plexin family are receptors for class 1 and V (viral) semaphorins. The present review will discuss a role for semaphorins and their receptors in the establishment and maintenance of olfactory connectivity.

2. Murakami Y, Suto F, Shimizu M, Shinoda T, Kameyama T, Fujisawa H. Differential expression of plexin-A subfamily members in the mouse nervous system. *Dev Dyn* 2001 Mar;220(3):246-58

Plexins comprise a family of transmembrane proteins (the plexin family) which are expressed in nervous tissues. Some plexins have been shown to interact directly with secreted

or transmembrane semaphorins, while plexins belonging to the A subfamily are suggested to make complexes with other membrane proteins, neuropilins, and propagate chemorepulsive signals of secreted semaphorins of class 3 into cells or neurons. Despite that much information has been gathered on the plexin-semaphorin interaction, the role of plexins in the nervous system is not well understood. To gain insight into the functions of plexins in the nervous system, we analyzed spatial and temporal expression patterns of three members of the plexin-A subfamily (plexin-A1, -A2, and -A3) in the developing mouse nervous system by in situ hybridization analysis in combination with immunohistochemistry. We show that the three plexins are differentially expressed in sensory receptors or neurons in a developmentally regulated manner, suggesting that a particular plexin or set of plexins is shared by neuronal elements and functions as the receptor for semaphorins to regulate neuronal development.

**Panel 2.2 Summary: Ag2011**

The expression of NOV12 appears to be highest in a sample derived from a melanoma metastasis. In addition, there is substantial expression in another melanoma sample. These results are in agreement with the results seen in Panel 1.3D, with significant expression detected in melanoma cell lines. Thus, the expression of this gene could be used to distinguish melanoma from other cancer types in this panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be of use in the treatment of melanoma.

**Panel 2D Summary: Ag1586**

The expression of NOV12 is highest in a sample derived from a metastasis of lung cancer. Thus, the expression of this gene could be used to distinguish this sample from the others in the panel. In addition, there is substantial expression in bladder cancer, when compared to its normal adjacent tissue, as well as in two samples of melanoma. Thus, the expression of this gene could be used to distinguish this bladder cancer from its normal adjacent tissue, or these melanomas from other samples. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be of use in the treatment of lung cancer, bladder cancer or melanoma.

**Panel 4D Summary: Ag2011**

Significant expression of the NOV12 transcript is found in small airway epithelium upon treatment with the pro-inflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  (CT= 26.5), the mucoid epidermoid cell line H 292 treated with IL-4 or IL-9, and in lung fibroblasts treated with IFN- $\gamma$  or IL-4. The constitutive expression of this transcript in these tissues is highly up-regulated by

pro-inflammatory cytokines or in conditions reflecting a Th2 mediated mechanism. Therefore, modulation of the expression of the protein encoded by this transcript could be useful for the treatment of lung inflammatory diseases that result from infection of the lung (bronchitis, pneumonia) and for the treatment of Th2-mediated lung disease such as asthma or COPD.

5 Significant expression of this transcript is also found in eosinophils upon PMA and ionomycin treatment, conditions that lead to production of eosinophil specific mediators. This production could contribute to the pathologies associated with asthma, other atopic diseases and inflammatory bowel disease. This gene encodes a novel protein with homology to members of the plexin family, a family of transmembrane proteins which act as receptors for semaphorins. 10 In neurons, semaphorins provide essential attractive and repulsive cues that are necessary for axon guidance. The description of the interaction of plexin with tyrosine kinase in the fetal lung suggests that this protein may play a role not only in morphogenesis but also in proliferation of activation. (See reference below.) Therefore, modulation of the expression of this protein by either antibody or small molecules could be beneficial for the treatment of 15 inflammatory lung, bowel and skin diseases.

Reference:

1. Cell 1999 Oct 1;99(1):71-80

Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates.

20 Tamagnone L, Artigiani S, Chen H, He Z, Ming GL, Song H, Chedotal A, Winberg ML, Goodman CS, Poo M, Tessier-Lavigne M, Comoglio PM.

Institute for Cancer Research and Treatment, University of Torino, Candiolo, Italy.  
ltamagnone@ircc.unito.it

In *Drosophila*, plexin A is a functional receptor for semaphorin-1a. Here we show that 25 the human plexin gene family comprises at least nine members in four subfamilies. Plexin-B1 is a receptor for the transmembrane semaphorin Sema4D (CD100), and plexin-C1 is a receptor for the GPI-anchored semaphorin Sema7A (Sema-K1). Secreted (class 3) semaphorins do not bind directly to plexins, but rather plexins associate with neuropilins, coreceptors for these semaphorins. Plexins are widely expressed: in neurons, the expression of a truncated plexin- 30 A1 protein blocks axon repulsion by Sema3A. The cytoplasmic domain of plexins associates with a tyrosine kinase activity. Plexins may also act as ligands mediating repulsion in epithelial cells in vitro. We conclude that plexins are receptors for multiple (and perhaps all)

classes of semaphorins, either alone or in combination with neuropilins, and trigger a novel signal transduction pathway controlling cell repulsion

PMID: 10520995

### Example 3. SNP analysis of NOVX clones

**SeqCalling™ Technology:** cDNA was derived from various human samples representing multiple tissue types, normal and diseased states, physiological states, and developmental states from different donors. Samples were obtained as whole tissue, cell lines, primary cells or tissue cultured primary cells and cell lines. Cells and cell lines may have been treated with biological or chemical agents that regulate gene expression for example, growth factors, chemokines, steroids. The cDNA thus derived was then sequenced using CuraGen's proprietary SeqCalling technology. Sequence traces were evaluated manually and edited for corrections if appropriate. cDNA sequences from all samples were assembled with themselves and with public ESTs using bioinformatics programs to generate CuraGen's human SeqCalling database of SeqCalling assemblies. Each assembly contains one or more overlapping cDNA sequences derived from one or more human samples. Fragments and ESTs were included as components for an assembly when the extent of identity with another component of the assembly was at least 95% over 50 bp. Each assembly can represent a gene and/or its variants such as splice forms and/or single nucleotide polymorphisms (SNPs) and their combinations.

Variant sequences are included in this application. A variant sequence can include a single nucleotide polymorphism (SNP). A SNP can, in some instances, be referred to as a "cSNP" to denote that the nucleotide sequence containing the SNP originates as a cDNA. A SNP can arise in several ways. For example, a SNP may be due to a substitution of one nucleotide for another at the polymorphic site. Such a substitution can be either a transition or a transversion. A SNP can also arise from a deletion of a nucleotide or an insertion of a nucleotide, relative to a reference allele. In this case, the polymorphic site is a site at which one allele bears a gap with respect to a particular nucleotide in another allele. SNPs occurring within genes may result in an alteration of the amino acid encoded by the gene at the position of the SNP. Intragenic SNPs may also be silent, however, in the case that a codon including a SNP encodes the same amino acid as a result of the redundancy of the genetic code. SNPs occurring outside the region of a gene, or in an intron within a gene, do not result in changes in any amino acid sequence of a protein but may result in altered regulation of the expression pattern for example, alteration in temporal expression, physiological response regulation, cell type expression regulation, intensity of expression, stability of transcribed message.

**Method of novel SNP Identification:** SNPs are identified by analyzing sequence assemblies using CuraGen's proprietary SNPTool algorithm. SNPTool identifies variation in assemblies with the following criteria: SNPs are not analyzed within 10 base pairs on both ends of an alignment; Window size (number of bases in a view) is 10; The allowed number of mismatches in a window is 2; Minimum SNP base quality (PHRED score) is 23; Minimum number of changes to score an SNP is 2/assembly position. SNPTool analyzes the assembly and displays SNP positions, associated individual variant sequences in the assembly, the depth of the assembly at that given position, the putative assembly allele frequency, and the SNP sequence variation. Sequence traces are then selected and brought into view for manual validation. The consensus assembly sequence is imported into CuraTools along with variant sequence changes to identify potential amino acid changes resulting from the SNP sequence variation. Comprehensive SNP data analysis is then exported into the SNPCalling database.

**Method of novel SNP Confirmation:** SNPs are confirmed employing a validated method known as Pyrosequencing (Pyrosequencing, Westborough, MA). Detailed protocols for Pyrosequencing can be found in: Alderborn et al. Determination of Single Nucleotide Polymorphisms by Real-time Pyrophosphate DNA Sequencing. (2000). *Genome Research*. 10, Issue 8, August. 1249-1265. In brief, Pyrosequencing is a real time primer extension process of genotyping. This protocol takes double-stranded, biotinylated PCR products from genomic DNA samples and binds them to streptavidin beads. These beads are then denatured producing single stranded bound DNA. SNPs are characterized utilizing a technique based on an indirect bioluminometric assay of pyrophosphate (PPi) that is released from each dNTP upon DNA chain elongation. Following Klenow polymerase-mediated base incorporation, PPi is released and used as a substrate, together with adenosine 5'-phosphosulfate (APS), for ATP sulfurylase, which results in the formation of ATP. Subsequently, the ATP accomplishes the conversion of luciferin to its oxi-derivative by the action of luciferase. The ensuing light output becomes proportional to the number of added bases, up to about four bases. To allow processivity of the method dNTP excess is degraded by apyrase, which is also present in the starting reaction mixture, so that only dNTPs are added to the template during the sequencing. The process has been fully automated and adapted to a 96-well format, which allows rapid screening of large SNP panels. The DNA and protein sequences for the novel single nucleotide polymorphic variants are reported. Variants are reported individually but any combination of all or a select subset of variants are also included. In addition, the positions of the variant bases and the variant amino acid residues are underlined.

### Results

Variants are reported individually but any combination of all or a select subset of variants are also included as contemplated NOVX embodiments of the invention.

#### NOV6 SNP data:

- 5 NOV6 has two SNP variants, whose variant positions for their nucleotide and amino acid sequences is numbered according to SEQ ID NOs:17 and 18, respectively. The nucleotide sequence of the NOV6 variants differs as shown in Table 81.

| Table 81. cSNP and Coding Variants for NOV6 |              |            |                     |                   |
|---------------------------------------------|--------------|------------|---------------------|-------------------|
| NT Position of cSNP                         | Wild Type NT | Variant NT | Amino Acid position | Amino Acid Change |
| 446                                         | T            | C          | No change           | No change         |
| 553                                         | A            | G          | No change           | No change         |

#### NOV8 SNP data:

- 10 NOV8 has two SNP variants, whose variant positions for their nucleotide and amino acid sequences is numbered according to SEQ ID NOs:21 and 22, respectively. The nucleotide sequence of the NOV8 variants differs as shown in Table 82.

| Table 82. cSNP and Coding Variants for NOV8 |              |            |                     |                   |
|---------------------------------------------|--------------|------------|---------------------|-------------------|
| NT Position of cSNP                         | Wild Type NT | Variant NT | Amino Acid position | Amino Acid Change |
| 564                                         | G            | A          | 109                 | G->D              |
| 976                                         | T            | G          | No change           | No change         |

#### NOV9 SNP data:

- 15 NOV 9 has two SNP variants, whose variant positions for their nucleotide and amino acid sequences is numbered according to SEQ ID NOs:23 and 24, respectively. The nucleotide sequence of the NOV9 variants differs as shown in Table 83.

| Table 83. cSNP and Coding Variants for NOV9 |              |            |                     |                   |
|---------------------------------------------|--------------|------------|---------------------|-------------------|
| NT Position of cSNP                         | Wild Type NT | Variant NT | Amino Acid position | Amino Acid Change |
| 111                                         | A            | C          | No change           | No change         |
| 200                                         | A            | G          | 62                  | K→R               |

#### NOV10 SNP data:

- 20 NOV10 has two SNP variants, whose variant positions for their nucleotide and amino acid sequences is numbered according to SEQ ID NOs:25 and 26, respectively. The nucleotide sequence of the NOV10 variants differs as shown in Table 84.

| Table 84. cSNP and Coding Variants for NOV10 |              |            |                     |                   |
|----------------------------------------------|--------------|------------|---------------------|-------------------|
| NT Position of cSNP                          | Wild Type NT | Variant NT | Amino Acid position | Amino Acid Change |
| 2129                                         | C            | T          | No change           | No change         |
| 2450                                         | T            | C          | No change           | No change         |

**NOV11 SNP data:**

NOV11a has three SNP variants, whose variant positions for their nucleotide and amino acid sequences is numbered according to SEQ ID NOs:27 and 28, respectively. The nucleotide sequence of the NOV11a variant differs as shown in Table 85.

| Table 85. cSNP and Coding Variants for NOV11a |              |            |                     |                   |
|-----------------------------------------------|--------------|------------|---------------------|-------------------|
| NT Position of cSNP                           | Wild Type NT | Variant NT | Amino Acid position | Amino Acid Change |
| 122                                           | C            | G          | No change           | No change         |
| 208                                           | G            | C          | No change           | No change         |
| 372                                           | C            | T          | 97                  | P->L              |
| 482                                           | A            | G          | 134                 | N->D              |

**Example 4. In-frame Cloning****NOV1b**

For NOV1b, the cDNA coding for the DOMAIN of NOV1a (CG50718-02) from residues 18 to 917 was targeted for "in-frame" cloning by PCR. The PCR template was based on the previously identified plasmid, when available, or on human cDNA(s).

**Table 86. Oligonucleotide primers used to clone the target cDNA sequence:**

| Primers | Sequences                                                 |
|---------|-----------------------------------------------------------|
| F1      | 5'-AGATCTCAGGTAGATGTTTCCAATGTCGTTCC-3' (SEQ ID NO:196)    |
| R1      | 5'-CTCGAGGCTAGCGTTACATAAGCACTGTATTCAAC-3' (SEQ ID NO:197) |

**NOV11c**

For NOV11c, the cDNA coding for the DOMAIN of NOV11b (CG54503\_02) from residues 15 to 238 was targeted for "in-frame" cloning by PCR. The PCR template was based on the previously identified plasmid, when available, or on human cDNA(s).

**Table 87. Oligonucleotide primers used to clone the target cDNA sequence:**

| Primers | Sequences                                                                |
|---------|--------------------------------------------------------------------------|
| F2      | 5'-GGATCC TCCGCGGGCCAGCGCACTACGAGATGCTGGGTCG-3' (SEQ ID NO:198)          |
| R1      | 5'-CTCGAGGTCGGGGTAGAT GATGAAGCCGGAGAAGGTGCTGTACTTGTGG-3' (SEQ ID NO:199) |

For downstream cloning purposes, the forward primer includes an in-frame Hind III restriction site and the reverse primer contains an in-frame Xho I restriction site.

Two parallel PCR reactions were set up using a total of 0.5-1.0 ng human pooled cDNAs as template for each reaction. The pool is composed of 5 micrograms of each of the following human tissue cDNAs: adrenal gland, whole brain, amygdala, cerebellum, thalamus, bone marrow, fetal brain, fetal kidney, fetal liver, fetal lung, heart, kidney, liver, lymphoma, Burkitt's Raji cell line, mammary gland, pancreas, pituitary gland, placenta, prostate, salivary gland, skeletal muscle, small Intestine, spleen, stomach, thyroid, trachea, uterus.

When the tissue of expression is known and available, the second PCR was performed using the above primers and 0.5ng-1.0 ng of one of the following human tissue cDNAs:

skeleton muscle, testis, mammary gland, adrenal gland, ovary, colon, normal cerebellum, normal adipose, normal skin, bone marrow, brain amygdala, brain hippocampus, brain substantia nigra, brain thalamus, thyroid, fetal lung, fetal liver, fetal brain, kidney, heart, spleen, uterus, pituitary gland, lymph node, salivary gland, small intestine, prostate, placenta, spinal cord, peripheral blood, trachea, stomach, pancreas, hypothalamus.

The reaction mixtures contained 2 microliters of each of the primers (original concentration: 5 pmol/ul), 1 microliter of 10mM dNTP (Clontech Laboratories, Palo Alto CA) and 1 microliter of 50xAdvantage-HF 2 polymerase (Clontech Laboratories) in 50 microliter-reaction volume. The following reaction conditions were used:

PCR condition 1:

- a) 96°C 3 minutes
- b) 96°C 30 seconds denaturation
- c) 60°C 30 seconds, primer annealing
- d) 72°C 6 minutes extension

Repeat steps b-d 15 times

- e) 96°C 15 seconds denaturation
- f) 60°C 30 seconds, primer annealing
- g) 72°C 6 minutes extension

Repeat steps e-g 29 times

- e) 72°C 10 minutes final extension

PCR condition 2:

- a) 96°C 3 minutes
- b) 96°C 15 seconds denaturation
- c) 76°C 30 seconds, primer annealing, reducing the temperature by 1 °C per cycle
- d) 72°C 4 minutes extension

Repeat steps b-d 34 times



e) 72°C 10 minutes final extension

An amplified product was detected by agarose gel electrophoresis. The fragment was gel-purified and ligated into the pCR2.1 vector (Invitrogen, Carlsbad, CA) following the manufacturer's recommendation. Twelve clones per PCR reaction were picked and sequenced. The inserts were sequenced using vector-specific M13 Forward and M13 Reverse primers and the gene-specific primers in Tables 88 and 89.

**Table 88. Gene-specific Primers**

| NOV    | Primers | Sequences                           |
|--------|---------|-------------------------------------|
| NOV11c | SF1     | GCCCTCCCGGTCCAGGTC (SEQ ID NO:200)  |
|        | SF2     | GGCGACGGCACCAGCATGT (SEQ ID NO:201) |
|        | SR1     | GCCTGGCCTGCCGGGTTCT (SEQ ID NO:202) |
|        | SR2     | CATGAGCACGTGGTAAGCG (SEQ ID NO:203) |

**Table 89. Gene-specific Primers**

| NOV   | Primers | Sequences                                     |
|-------|---------|-----------------------------------------------|
| NOV1b | SF1     | GTGCTGGCATTGGAGTGTTTAGTG (SEQ ID NO:204)      |
|       | SF2     | ATCAAGCACGTTGACACAGAATGAG (SEQ ID NO:205)     |
|       | SF3     | GCATTCACTAACCTAACACCATTTACA (SEQ ID NO:206)   |
|       | SF4     | GTTGAGCAGAGATGTCGTCTGACCTTC (SEQ ID NO:207)   |
|       | SF5     | GGGATCCTCCAGATCCTGTATTTT (SEQ ID NO:208)      |
|       | SF6     | TGAAGAACACATCAACAACAGACATAA (SEQ ID NO:209)   |
|       | SR1     | ACTGTTTTTCAGCAGCTACCTTAATTC (SEQ ID NO:210)   |
|       | SR2     | CTTGATGAATGTGTGGTACGCGAT (SEQ ID NO:211)      |
|       | SR3     | GTGAATGCAAACCTGAGGTCTTTTGT (SEQ ID NO:212)    |
|       | SR4     | CCTCATATAATCCTACCATTGGCTGTACT (SEQ ID NO:213) |
|       | SR5     | GAGGATCCCAGTGTAATAATACTTCTG (SEQ ID NO:214)   |
|       | SR6     | TAGCACTTCATAAGCAATAATGATCCC (SEQ ID NO:215)   |
|       | SR7     | TGAGTGTAAGTAGCAGACACCTCAATGAT (SEQ ID NO:216) |

### OTHER EMBODIMENTS

Although particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims, which follow. In particular, it is  
5 contemplated by the inventors that various substitutions, alterations, and modifications may be made to the invention without departing from the spirit and scope of the invention as defined by the claims. The choice of nucleic acid starting material, clone of interest, or library type is believed to be a matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described herein. Other aspects, advantages, and modifications considered to be  
10 within the scope of the following claims.

**WHAT IS CLAIMED IS:**

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
  - (a) a mature form of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32;
  - (b) a variant of a mature form of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of the amino acid residues from the amino acid sequence of said mature form;
  - (c) an amino acid sequence selected from the group consisting SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32; and
  - (d) a variant of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of amino acid residues from said amino acid sequence.
2. The polypeptide of claim 1, wherein said polypeptide comprises the amino acid sequence of a naturally-occurring allelic variant of an amino acid sequence selected from the group consisting SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32.
3. The polypeptide of claim 2, wherein said allelic variant comprises an amino acid sequence that is the translation of a nucleic acid sequence differing by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, and 31.
4. The polypeptide of claim 1, wherein the amino acid sequence of said variant comprises a conservative amino acid substitution.

5. An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence selected from the group consisting of:
- (a) a mature form of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32;
  - (b) a variant of a mature form of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of the amino acid residues from the amino acid sequence of said mature form;
  - (c) an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32;
  - (d) a variant of an amino acid sequence selected from the group consisting SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of amino acid residues from said amino acid sequence;
  - (e) a nucleic acid fragment encoding at least a portion of a polypeptide comprising an amino acid sequence chosen from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32, or a variant of said polypeptide, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of amino acid residues from said amino acid sequence; and
  - (f) a nucleic acid molecule comprising the complement of (a), (b), (c), (d) or (e).
6. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises the nucleotide sequence of a naturally-occurring allelic nucleic acid variant.
7. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule encodes a polypeptide comprising the amino acid sequence of a naturally-occurring polypeptide variant.

8. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule differs by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, and 31.
9. The nucleic acid molecule of claim 5, wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of:
  - (a) a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, and 31;
  - (b) a nucleotide sequence differing by one or more nucleotides from a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, and 31, provided that no more than 20% of the nucleotides differ from said nucleotide sequence;
  - (c) a nucleic acid fragment of (a); and
  - (d) a nucleic acid fragment of (b).
10. The nucleic acid molecule of claim 5, wherein said nucleic acid molecule hybridizes under stringent conditions to a nucleotide sequence chosen from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, and 31, or a complement of said nucleotide sequence.
11. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of:
  - (a) a first nucleotide sequence comprising a coding sequence differing by one or more nucleotide sequences from a coding sequence encoding said amino acid sequence, provided that no more than 20% of the nucleotides in the coding sequence in said first nucleotide sequence differ from said coding sequence;
  - (b) an isolated second polynucleotide that is a complement of the first polynucleotide; and
  - (c) a nucleic acid fragment of (a) or (b).
12. A vector comprising the nucleic acid molecule of claim 11.
13. The vector of claim 12, further comprising a promoter operably-linked to said nucleic acid molecule.

14. A cell comprising the vector of claim 12.
15. An antibody that binds immunospecifically to the polypeptide of claim 1.
16. The antibody of claim 15, wherein said antibody is a monoclonal antibody.
17. The antibody of claim 15, wherein the antibody is a humanized antibody.
18. A method for determining the presence or amount of the polypeptide of claim 1 in a sample, the method comprising:
  - (a) providing the sample;
  - (b) contacting the sample with an antibody that binds immunospecifically to the polypeptide; and
  - (c) determining the presence or amount of antibody bound to said polypeptide,thereby determining the presence or amount of polypeptide in said sample.
19. A method for determining the presence or amount of the nucleic acid molecule of claim 5 in a sample, the method comprising:
  - (a) providing the sample;
  - (b) contacting the sample with a probe that binds to said nucleic acid molecule; and
  - (c) determining the presence or amount of the probe bound to said nucleic acid molecule,thereby determining the presence or amount of the nucleic acid molecule in said sample.
20. The method of claim 19 wherein presence or amount of the nucleic acid molecule is used as a marker for cell or tissue type.
21. The method of claim 20 wherein the cell or tissue type is cancerous.
22. A method of identifying an agent that binds to a polypeptide of claim 1, the method comprising:
  - (a) contacting said polypeptide with said agent; and
  - (b) determining whether said agent binds to said polypeptide.

23. The method of claim 22 wherein the agent is a cellular receptor or a downstream effector.
24. A method for identifying an agent that modulates the expression or activity of the polypeptide of claim 1, the method comprising:
- (a) providing a cell expressing said polypeptide;
  - (b) contacting the cell with said agent, and
  - (c) determining whether the agent modulates expression or activity of said polypeptide,
- whereby an alteration in expression or activity of said peptide indicates said agent modulates expression or activity of said polypeptide.
25. A method for modulating the activity of the polypeptide of claim 1, the method comprising contacting a cell sample expressing the polypeptide of said claim with a compound that binds to said polypeptide in an amount sufficient to modulate the activity of the polypeptide.
26. A method of treating or preventing a NOVX-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired the polypeptide of claim 1 in an amount sufficient to treat or prevent said NOVX-associated disorder in said subject.
27. The method of claim 26 wherein the disorder is selected from the group consisting of cardiomyopathy and atherosclerosis.
28. The method of claim 26 wherein the disorder is related to cell signal processing and metabolic pathway modulation.
29. The method of claim 26, wherein said subject is a human.
30. A method of treating or preventing a NOVX-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired

the nucleic acid of claim 5 in an amount sufficient to treat or prevent said NOVX-associated disorder in said subject.

31. The method of claim 30 wherein the disorder is selected from the group consisting of cardiomyopathy and atherosclerosis.
32. The method of claim 30 wherein the disorder is related to cell signal processing and metabolic pathway modulation.
33. The method of claim 30, wherein said subject is a human.
34. A method of treating or preventing a NOVX-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired the antibody of claim 15 in an amount sufficient to treat or prevent said NOVX-associated disorder in said subject.
35. The method of claim 34 wherein the disorder is diabetes.
36. The method of claim 34 wherein the disorder is related to cell signal processing and metabolic pathway modulation.
37. The method of claim 34, wherein the subject is a human.
38. A pharmaceutical composition comprising the polypeptide of claim 1 and a pharmaceutically-acceptable carrier.
39. A pharmaceutical composition comprising the nucleic acid molecule of claim 5 and a pharmaceutically-acceptable carrier.
40. A pharmaceutical composition comprising the antibody of claim 15 and a pharmaceutically-acceptable carrier.
41. A kit comprising in one or more containers, the pharmaceutical composition of claim 38.



42. A kit comprising in one or more containers, the pharmaceutical composition of claim 39.
43. A kit comprising in one or more containers, the pharmaceutical composition of claim 40.
44. A method for determining the presence of or predisposition to a disease associated with altered levels of the polypeptide of claim 1 in a first mammalian subject, the method comprising:
- (a) measuring the level of expression of the polypeptide in a sample from the first mammalian subject; and
  - (b) comparing the amount of said polypeptide in the sample of step (a) to the amount of the polypeptide present in a control sample from a second mammalian subject known not to have, or not to be predisposed to, said disease;

wherein an alteration in the expression level of the polypeptide in the first subject as compared to the control sample indicates the presence of or predisposition to said disease.

45. The method of claim 44 wherein the predisposition is to a cancer.
46. A method for determining the presence of or predisposition to a disease associated with altered levels of the nucleic acid molecule of claim 5 in a first mammalian subject, the method comprising:
- (a) measuring the amount of the nucleic acid in a sample from the first mammalian subject; and
  - (b) comparing the amount of said nucleic acid in the sample of step (a) to the amount of the nucleic acid present in a control sample from a second mammalian subject known not to have or not be predisposed to, the disease;
- wherein an alteration in the level of the nucleic acid in the first subject as compared to the control sample indicates the presence of or predisposition to the disease.
47. The method of claim 46 wherein the predisposition is to a cancer.

48. A method of treating a pathological state in a mammal, the method comprising administering to the mammal a polypeptide in an amount that is sufficient to alleviate the pathological state, wherein the polypeptide is a polypeptide having an amino acid sequence at least 95% identical to a polypeptide comprising an amino acid sequence of at least one of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32, or a biologically active fragment thereof.
49. A method of treating a pathological state in a mammal, the method comprising administering to the mammal the antibody of claim 15 in an amount sufficient to alleviate the pathological state.